Sepsis-induced muscle growth hormone resistance occurs independently of STAT5 phosphorylation

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Hong-Brown, Ly Q., C. Randell Brown, Robert N. Cooney, Robert A. Frost, and Charles H. Lang. Sepsis-induced muscle growth hormone resistance occurs independently of STAT5 phosphorylation. Am J Physiol Endocrinol Metab 285: E63–E72, 2003. First published March 18, 2003; 10.1152/ajpendo.00555.2002.—Growth hormone (GH) stimulates insulin-like growth factor I (IGF-I) synthesis in both liver and muscle. During sepsis, proinflammatory cytokines inhibit GH action in liver, but it is unknown whether sepsis also produces GH resistance in muscle. Sepsis was induced by cecal ligation and puncture, and 18 h later the effect of GH on signal transducer and activator of transcription (STAT) phosphorylation and IGF-I mRNA content was assessed in rat gastrocnemius and liver. The relative abundance of phosphorylated (p)STAT5a, pSTAT5b, pSTAT3, and pSTAT1 was increased in liver from control rats after GH. Sepsis alone also increased hepatic pSTAT5a, pSTAT3, and pSTAT1. Sepsis dramatically impaired the ability of GH to stimulate the phosphorylation of STAT5a and -5b, as well as to increase IGF-I mRNA in liver. In muscle from control rats, GH increased pSTAT5a and -5b, whereas content of pSTAT3 and pSTAT1 was not affected. Sepsis increased basal content of pSTAT3 but not pSTAT5a, pSTAT5b, or pSTAT1 in muscle. The GH-induced increase of pSTAT5a and -5b in muscle from septic rats was not inhibited, suggesting that muscle was not GH resistant. In contrast to these changes in pSTAT5, the ability of GH to increase IGF-I mRNA was completely absent in muscle from septic rats. Because the suppressor of cytokine signaling (SOCS) proteins may function as negative regulators of GH signaling, we examined the content of these proteins. Sepsis produced small (30–50%), albeit statistically significant, increases in SOCS-1, -2, and -3 protein in muscle. In contrast to muscle, the SOCS proteins in the liver did not change under the various experimental conditions, suggesting that these proteins are not responsible for the impaired phosphorylation of STAT5 by GH. In conclusion, sepsis produces GH resistance in both muscle and liver, with the locus of this impairment in muscle differing from that in liver and being independent of a defect in STAT5 phosphorylation.

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GROWTH HORMONE (GH) receptor binding initiates a number of well-characterized signaling events, including receptor dimerization and the subsequent auto-phosphorylation of the nonreceptor tyrosine kinase Janus kinase (JAK)2 and JAK3 (21, 47). GH stimulates the tyrosine phosphorylation of several signaling proteins, including insulin receptor substrate-1, -2, and -3 (4, 45), the Shc protein (39, 42), and the signal transducer and activator of transcription (STAT) proteins STAT1, STAT3, STAT5a, and STAT5b (7, 19–21, 31). STAT5a and STAT5b are highly homologous proteins that are phosphorylated in the presence of GH and cytokines. This phosphorylation leads to their dimerization and nuclear translocation, where they modulate gene transcription. Despite their similarities, the STAT5a and STAT5b proteins exhibit distinct functions. Mice with a deletion of both the STAT5a and STAT5b genes display growth retardation in both sexes, with males exhibiting a more profound effect (38). This response appears to be due to a lack of STAT5b, because STAT5a-deficient mice are indistinguishable from wild-type littermates in terms of size and weight. Importantly, STAT5b plays an important role in hepatic insulin-like growth factor I (IGF-I) gene expression in hypophysectomized mice (12).

A decrease in the circulating concentration of IGF-I has been reported in response to endotoxin [e.g., lipopolysaccharide (LPS)] and sepsis (11, 33). Furthermore, this change is associated with decreased abundance of IGF-I mRNA and protein in liver and muscle (23, 29). Although the mechanism for this inflammation-induced decrease in IGF-I is poorly understood, it has been proposed to be responsible, at least in part, for the muscle wasting produced by trauma and critical illness (15).

Several lines of evidence suggest that LPS and sepsis lead to the development of hepatic GH resistance. First, in humans injected with LPS there is an increase in the plasma GH concentration and a concomitant reduction in circulating IGF-I (28). Second, the ability of GH to increase plasma IGF-I in septic patients is markedly attenuated (10). Third, treatment of hepatocytes with inflammatory cytokines blunts the GH-induced increase in IGF-I mRNA (40, 46). Fourth, previous studies have demonstrated that LPS blunts GH-
induced phosphorylation of STAT5 in liver (30). Finally, the suppressors of cytokine signaling (SOCS)-1–7 and CIS genes are upregulated by a number of inflammatory cytokines, and the presence of specific cytokines potentiates the GH-induced increase in SOCS expression in cultured hepatocytes (9). The overexpression of SOCS proteins has been shown to inhibit GH action by downregulating various aspects of the Janus kinase (JAK)/STAT signaling pathway, such as the interaction of the GH receptor with JAKs as well as JAK tyrosine kinase activity (14, 32).

Although several studies have examined the mechanisms leading to sepsis-induced GH resistance (13, 30), all of the studies have been conducted using liver tissue. Consequently, the importance of GH resistance in other tissues, such as muscle, during systemic infection is unclear at present. In the current study, we determined the effect of sepsis on GH-induced changes in STAT phosphorylation and steady-state IGF-I mRNA content in rat muscle, and we compared the changes to those observed in liver. The tissue content of various SOCS proteins was also assessed as a possible mechanism for the development of GH resistance. These studies were designed to test the hypothesis that systemic infection inhibits the phosphorylation of STAT5 by GH, thereby diminishing GH-induced increases in muscle IGF-I mRNA.

MATERIALS AND METHODS

Reagents. Recombinant human GH was provided by Genentech (South San Francisco, CA), and Escherichia coli LPS was obtained from Difco (Detroit, MI). Antibodies to phosphorylated STAT1 (Tyr701) and STAT3 (Tyr705), as well as antibodies to STAT1, STAT3, STAT5a, STAT5b, SOCS-1, -2, -3, CIS, and the protein inhibitor of activated STAT1 and STAT3 (PIAS), were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); the phosphospecific STAT5 (Tyr694) antibody was from Cell Signaling Technology (Beverly, MA). Protein A- and protein G-Sepharose beads were obtained from Pharmacia (Piscataway, NJ), and pentobarbital sodium was purchased from Abbott Laboratories (Chicago, IL). All other reagents were purchased from Sigma Chemical (St. Louis, MO).

Animals. Male Sprague-Dawley rats weighing 200–300 g were purchased from Charles River Breeding Laboratories (Cambridge, MA). Rats were acclimated for 1 wk in a light-controlled room (12:12-h light-dark cycle) under constant temperature. Water and standard rat chow were provided ad libitum. All experiments were approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine and adhered to the National Institutes of Health (NIH) guidelines for the use of experimental animals.

Experimental protocols. For experiments in which sepsis was induced by cecal ligation and puncture (CLP), rats were anesthetized with pentobarbital sodium and midline laparotomy was performed. The cecum was ligated at its base and punctured twice using a 20-gauge needle. The cecum was then returned to the peritoneal cavity, and the muscle and skin layers were closed. Rats were resuscitated with 10 ml of 0.9% sterile saline administered subcutaneously. Control animals were subjected to a midline laparotomy with intestinal manipulation and then were resuscitated with the same volume of saline. During the operation, rats were placed on a warming pad to maintain body temperature. After surgery, food was withheld, but the animals were permitted free access to water for the remainder of the study. Hence, any observed changes between septic and control rats cannot be attributed to differences in food intake or nutritional status.

Approximately 18 h after induction of sepsis, rats were anesthetized with pentobarbital, and a midline laparotomy was performed. A single dosage of GH (1.5 mg/kg) or an equal volume of vehicle (0.5 ml of 0.9% saline containing 0.2% bovine serum albumin) was injected into the inferior vena cava. This GH dose has been reported to maximally activate the JAK/STAT-signaling pathway (8, 30) and to increase tissue content of IGF-I mRNA and protein (26). The animals were killed 20 min after GH administration, a time for which our preliminary studies demonstrated a maximal increase in STAT5 phosphorylation in muscle.

In a second experimental series, animals were injected intraperitoneally with either LPS (1 mg/kg) or an equal volume of saline (0.5 ml/kg). This dose of LPS was selected on the basis of previous studies demonstrating its ability to produce hepatic GH resistance (29, 30) and decrease IGF-I mRNA in both muscle and liver. Four hours after LPS administration, rats were anesthetized with pentobarbital and injected intravenously with either GH or saline, as described above. Animals were then killed 20 min after GH injection. For the first two experimental protocols, gastrocnemius muscle and liver were frozen between liquid nitrogen-cooled aluminum clamps at the time animals were killed.

A third experimental series was performed, in which control and septic rats received two injections of GH. For this study, GH was administered subcutaneously to conscious unrestrained rats at a dose of 1.5 mg/kg. The first injection was administered 4 h after the CLP procedure, and the second injection was given 8 h thereafter. Rats were killed 12 h after the second GH injection, and the plasma IGF-I concentration and abundance of IGF-I mRNA in liver and gastrocnemius were determined. Tissues from this group were used solely for the determination of IGF-I mRNA content because of the transient nature of STAT phosphorylation. The total IGF-I concentration in plasma was determined by radioimmunoassay after acid-ethanol extraction and cryoprecipitation, as previously described (24). Tissues were removed, freeze-clamped, and stored at −70°C for subsequent analysis.

Immunoprecipitation and immunoblotting. Frozen muscle or liver tissues were weighed, pulverized under liquid nitrogen, and homogenized with a polytron in 4 volumes of lysate buffer containing 50 mM Tris (pH 7.4), 1% Triton, 0.25% Na-deoxycholate, 1 mM EDTA, and a cocktail of protease and phosphatase inhibitors (Sigma). The homogenates were clarified by centrifugation at 10,000 rpm for 20 min and stored at −70°C before analysis. To determine the tissue content of various STAT and SOCS proteins, homogenates were separated by SDS-PAGE and then subjected to Western blot analysis. The phosphorylated forms of these proteins were determined by immunoprecipitating with the same antibodies, followed by Western blot analysis using a phosphotyrosine-specific antibody. For immunoprecipitation, samples were incubated with specific antibodies and rotated overnight at 4°C. The resulting immunocomplexes were then absorbed onto protein A- or protein G-Sepharose beads for 1–2 h. After two washings, bound protein was eluted by boiling in Laemmli sample buffer. Immunoprecipitates and total cell lysates were resolved by SDS-PAGE (10 and 15%) and electrophoresed onto nitrocellulose membranes, which were blocked with 5% nonfat dry milk and incubated with the indicated antibodies. After a wash with Tris-buffered saline
containing 0.05% Tween-20, the blots were incubated with secondary antibodies conjugated with horseradish peroxidase. Blots were then incubated with an enhanced chemiluminescent detection system (Amersham, Buckinghamshire, UK) and exposed to X-ray film. The film was scanned (Microtek ScanMaker 4, Carson, CA) and quantified using NIH Image 1.6 software.

RNA extraction. Total RNA from tissue was extracted using TriReagent (Molecular Research Center, Cincinnati, OH). Total RNA was quantified by measuring the absorbance at 260 nm. Samples (20 μg) of muscle or liver total RNA were run under denaturing conditions in 1% agarose-6% formaldehyde gels with a 1× HEPES running buffer. Northern blotting occurred via capillary transfer to Nytran SuperCharge membranes (Schleicher and Schuell, Keene, NH), as previously described (27). An 800-bp probe from rat IGF-I (from Peter Rotwein of Portland, OR) was labeled using a Random Primed DNA Labeling kit (Roche Molecular Biochemicals, Indianapolis, IN). For normalization of RNA loading, a rat 18S oligonucleotide was radioactively labeled using TdT (Promega, Madison, WI). Northern blots were hybridized using ULTRAhyb (Ambion, Austin, TX). All membranes were initially washed twice in 0.2× SSC-0.1% SDS for 5 min at 42°C. 18S was then washed in 0.2× SSC-0.1% SDS for 15 min at 48°C and for 15 min at 65°C. Finally, membranes were exposed to a phosphorimager screen, and the resultant data were quantified using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Statistical analysis. Statistical significance was determined using one-way ANOVA followed by the Student-Neuman-Keuls test. Data are presented as means ± SE. Mean values were considered to be significantly different at \( P < 0.05 \).

RESULTS

STAT5 tyrosine phosphorylation in muscle and liver of septic rats. To determine whether CLP-induced sepsis affects GH-stimulated tyrosine phosphorylation of STAT5a in muscle, tissue extracts were immunoprecipitated with STAT5a antibody, followed by immunoblotting with phosphotyrosine-specific STAT5 (pSTAT5) antibody. The same immunoprecipitates were also blotted with STAT5a antibody for determination of the total content of this protein. Sepsis did not alter the basal (e.g., unstimulated) amount of pSTAT5a in gastrocnemius (Fig. 1A). GH increased pSTAT5a ~60% in control rats compared with basal values from the same group. In septic animals, GH increased pSTAT5a >2.5-fold compared with basal values from septic rats. The GH-induced increase in STAT5a phosphorylation in septic rats was significantly (~85%) greater than the GH-induced increase in control rats. Stimulation of STAT5a phosphorylation by GH was not due to an increase in total STAT5a protein (Fig. 1A, inset). Collectively, these results indicate that sepsis does not impair the ability of GH to stimulate STAT5a phosphorylation in a representative fast-twitch skeletal muscle.

In liver, an approximately sixfold increase in STAT5a tyrosine phosphorylation was detected after GH stimulation in control animals (Fig. 1B). Sepsis also modestly increased hepatic pSTAT5a under basal conditions, in contrast to our results from muscle. GH stimulation did not significantly increase hepatic pSTAT5a in septic rats. This sepsis-induced attenuation of GH action was not due to changes in total STAT5a protein (Fig. 1B, inset). Thus sepsis-induced GH resistance, at least at the level of STAT5a phosphorylation, appears to be a characteristic of liver but not skeletal muscle.

Although STAT5a and STAT5b are highly homologous, they may have differential effects on cellular function. Therefore, we next examined whether STAT5b responded to GH and sepsis in a manner comparable to that of STAT5a. Sepsis had no effect on the phosphorylation of STAT5b in muscle under basal conditions. However, pSTAT5b was increased about twofold by GH in muscle from control rats (Fig. 2A). After CLP, GH increased the phosphorylation of STAT5b about threefold compared with basal values from septic rats. Hence, sepsis appeared to potentiate...
the GH-induced increase in STAT5b phosphorylation in skeletal muscle. In liver, GH increased tyrosine phosphorylation of STAT5b \( \times 3.5 \) fold compared with basal values (Fig. 2B). Once again, this effect was markedly attenuated in septic animals. Hence, the sepsis-induced impairment in STAT5a and -5b phosphorylation in liver appears coordinately regulated.

The bolus injection of LPS produces an acute stress response that is more severe than that produced by CLP-induced sepsis. Therefore, we also assessed whether acute endotoxemia might be of sufficient severity to impair GH stimulation of STAT5 phosphorylation in muscle. LPS failed to significantly alter the basal content of pSTAT5b in gastrocnemius (Fig. 3A). As observed following CLP, GH stimulated the phosphorylation of STAT5b in muscle from both control and LPS-treated rats, but to a greater extent in animals receiving LPS. In contrast, decreased STAT5 phosphorylation was observed in liver tissue from LPS-treated rats after GH stimulation. A similar response to GH was observed when pSTAT5a was assessed in muscle and liver (data not shown). These data extend observations from the first experimental protocol and indicate that LPS also does not impair GH phosphorylation of STAT5 in muscle.

In liver, the STAT3 and STAT1 proteins can be activated by either GH or the administration of LPS (19, 20, 34). However, the effects of sepsis on STAT1 and STAT3 phosphorylation in muscle after GH administration have not been previously elucidated. GH did not significantly alter pSTAT3 in muscle from control rats (Fig. 4A). However, sepsis increased muscle pSTAT3 by \( \sim 95\% \). The content of pSTAT3 was similarly increased in septic rats injected with GH. The increased STAT3 phosphorylation observed in septic rats resulted in part from the increase in total STAT3 protein. Therefore, in muscle, there were no statistically significant differences between groups when values for pSTAT3 were normalized to total STAT3; control = 100 ± 8 arbitrary units (AU), control + GH = 103 ± 7 AU, septic = 131 ± 40 AU, and septic + GH = 134 ± 16 AU. In contrast to muscle, hepatic pSTAT3...
was increased ~60% after GH in control rats (Fig. 4B). However, sepsis alone increased hepatic pSTAT3 more than sevenfold above time-matched basal control values. A comparable increase in hepatic pSTAT3 was observed in GH-injected septic rats. Although a portion of the sepsis-induced increase in hepatic pSTAT3 could be accounted for by the two- to threefold elevation in total STAT3, the relative abundance of pSTAT3 was still significantly increased above control values when these data were normalized for total STAT3. The amount of pSTAT3 normalized for total STAT3 was for control 100 ± 5 AU, control + GH 156 ± 20 AU, septic 326 ± 34 AU, and septic + GH 414 ± 55 AU (P < 0.05, control vs. septic).

In gastrocnemius, neither sepsis nor GH significantly altered pSTAT1 content (Fig. 5A). In contrast, GH produced a 55% increase in hepatic pSTAT1 in control rats, and this response could not be explained by a change in the total amount of STAT1. Under basal septic conditions, hepatic pSTAT1 was also increased by 70% compared with values from control animals (Fig. 5B). GH stimulation of hepatic pSTAT1 in septic rats was increased ~50%, but this change failed to achieve statistical significance. Collectively, these sepsis- and GH-induced changes in pSTAT1 were of much smaller magnitude than those changes observed for STAT3 or STAT5 phosphorylation in liver, and their physiological importance is uncertain.

Sepsis-induced changes in SOCS protein. A number of inhibitory proteins, such as SOCS and PIAS, have been suggested to terminate GH-activated STAT signaling within cells (9, 18). Therefore, we examined whether the protein content of these regulators was altered in response to sepsis. In muscle, sepsis increased SOCS-1, SOCS-2, and SOCS-3 protein compared with basal values from control rats (Fig. 6, A, B, and C, respectively). Although these sepsis-induced changes were small, averaging 30–50% above control values, they were a consistent finding and achieved
statistical significance. There was no statistically significant difference in the PIAS and CIS protein content in muscle between septic and control rats (data not shown). In the liver, the content of SOCS, PIAS, and CIS inhibitor proteins was not altered 18 h after induction of sepsis (data not shown).

**GH effect on IGF-I mRNA.** The above-mentioned data imply that skeletal muscle of septic rats is not resistant to the stimulatory effects of GH, at least at the level of STAT5 phosphorylation. Therefore, we next examined the induction of IGF-I mRNA expression, a physiologically relevant endpoint, by GH in muscle from septic rats. In control rats, GH significantly increased IGF-I mRNA content in muscle (60%) and liver (30%) compared with basal values from control rats (Fig. 7, B and C, respectively). These GH-induced increases in tissue IGF-I mRNA were associated with a 55% increase in the plasma IGF-I concentration in control rats (Fig. 7A). Sepsis decreased IGF-I mRNA in both tissues (55 and 30%, respectively) as well as the circulating concentration of the peptide (40%). It is noteworthy that GH was unable to increase either the plasma IGF-I concentration or the IGF-I mRNA content in either muscle or liver from septic rats. In contrast to the data pertaining to GH stimulation of STAT5 phosphorylation, these data suggest that sepsis produces a GH-resistant state in both skeletal muscle and liver when IGF-I is used as the end point.

The content of SOCS proteins was also assessed in tissues from rats injected twice with GH (e.g., those rats used for IGF-I mRNA determination). There was no sepsis-induced change in any of these proteins in liver or skeletal muscle at this time point (data not shown).

**DISCUSSION**

GH rapidly and transiently activates a number of intracellular signaling pathways, including the phosphorylation of STAT proteins by JAKs (36). Previous
studies have shown that LPS and inflammatory cytokines inhibit hepatic GH signaling by impairing the phosphorylation and nuclear translocation of STAT5 (6, 30, 46). However, the effects of sepsis on GH signaling in skeletal muscle have not previously been examined. Because of the known importance of GH action in muscle and the regulatory role played by locally synthesized IGF-I, this lack of information regarding GH action in muscle limits understanding of mechanisms mediating the erosion of lean body mass in various catabolic conditions. Furthermore, most studies investigating selective aspects of GH signal transduction have failed to correlate the observed changes with a physiologically relevant end point, such as increase in IGF-I mRNA.

In liver from control rats, GH stimulated the phosphorylation of STAT5a and -5b, and to a lesser extent STAT3 and STAT1. These results, in general, confirm in vivo observations in liver by other investigators (5, 8, 17–20). Similarly, 24 h after induction of peritonitis by CLP, hepatic content of phosphorylated STAT5a, STAT3, and STAT1 was elevated. Of these three STATs, the sepsis-induced increase in pSTAT3 (7-fold) was the most dramatic. Although a portion of this increase could be attributed to the corresponding two-fold increase in total STAT3, sepsis clearly increased the percentage of total STAT3 in the active phosphorylated form. This elevation is consistent with previous data indicating that sepsis increases STAT3 DNA-binding activity in liver nuclear extracts (3). In addition, the relatively smaller increases in pSTAT5 and pSTAT1 induced by sepsis extend observations from LPS-treated animals (34). Finally, sepsis induces hepatic GH resistance, as evidenced by the failure of GH to stimulate phosphorylation of STAT5a and -5b. A defect in hepatic GH-stimulated STAT5 phosphorylation has been reported in liver from endotoxemic rats (6, 30) and in hepatocytes treated with TNF-α (46).

STAT5b is the predominant isoform in liver and appears to have an essential role in hepatic synthesis of IGF-I, as evidenced by the inability of GH to increase IGF-I mRNA expression in STAT5b-null mice (12). Hence, the defect in STAT5b phosphorylation in septic rats would be expected to impair the ability of GH to increase hepatic IGF-I mRNA. Moreover, because ~80% of the blood-borne IGF-I is derived from liver (44), this hepatic GH resistance should also be manifested by a smaller increase in plasma IGF-I in response to GH stimulation. In the current study, sepsis decreased hepatic IGF-I mRNA and the circulating concentration of IGF-I under basal conditions. Furthermore, sepsis prevented the induction of hepatic IGF-I mRNA and the increase in plasma IGF-I by GH. Collectively, these data confirm that sepsis produces hepatic GH resistance and demonstrate for the first time that impaired STAT signaling is associated with a physiologically significant inhibition of IGF-I synthesis by GH.

The characteristically observed decrease in plasma IGF-I is believed to be in part responsible for the muscle wasting observed in sepsis and other catabolic conditions (25). However, the ability of GH to stimulate the synthesis of IGF-I within muscle per se may also have an important role in maintaining normal protein accretion (44). Hence, the presence of impaired GH action in muscle, similar to that observed in liver, would represent a potentially important metabolic defect. In control rats, GH increased both pSTAT5a and -5b in skeletal muscle, a response that was qualitatively similar but quantitatively smaller than the response observed in liver. In contradistinction to liver, GH-induced phosphorylation of pSTAT5a and -5b was greater in skeletal muscle from septic rats than in muscle from control animals. To confirm and extend this observation, a second experimental protocol was performed, in which rats received a relatively high dose of LPS, which produces an acute catabolic condition characterized by the massive overexpression of a wide variety of inflammatory mediators. Similar to CLP, there was no impairment in the ability of GH to stimulate the phosphorylation of STAT5a and -5b in muscle from LPS-treated rats. Collectively, these data clearly indicate that, at the level of this particular signaling component, sepsis and the related insult produced by LPS do not produce GH resistance in skeletal muscle. Hence, the differential tissue response to GH is unlikely to be due to differences in the experimental models. Finally, the ability of GH to stimulate the phosphorylation of STAT5 was also not impaired in cardiac muscle from septic rats (data not shown), suggesting that GH resistance may be a liver-specific, not muscle-specific, phenomenon.

On the basis of the above-mentioned data, we anticipated that GH would increase muscle IGF-I mRNA to the same extent in both septic and control rats. Therefore, the complete absence of a GH-induced increase of IGF-I mRNA in muscle of septic rats was unexpected. The exact mechanism for this mismatch between STAT5 phosphorylation and IGF-I mRNA in muscle of septic rats was unclear, but several possibilities exist. First, it is possible that phosphorylation of STAT5 does not exert the same regulatory influence on IGF-I transcription in muscle as in liver. Previous work using cultured myocytes has demonstrated that, whereas GH stimulates both STAT5 and STAT3 phosphorylation, selective inhibition of STAT3 phosphorylation prevents the normally observed increase in IGF-I mRNA (16). The importance of such a pathway in vivo is questionable, because we were unable to detect a GH-induced increase in muscle pSTAT3 in either control or septic rats. Another possibility is that the GH-signaling pathways responsible for IGF-I transcription in muscle are independent of STAT5 phosphorylation. Second, phosphorylated STAT5 may not translocate to the nucleus in muscle and is not available to stimulate gene transcription. Third, STAT5 may increase IGF-I mRNA transcription, but steady-state content of IGF-I mRNA remains unchanged because of a corresponding decrease in the stability of the message. Finally, it is important to recognize that the determinations of STAT5 phosphorylation and IGF-I mRNA content were made at two different time points. This difference was necessitated...
by the inherently rapid and transient increase in STAT phosphorylation and the ability to detect increases in IGF-I mRNA only at relatively later time points in rats with functioning pituitary glands.

In the current study, the regulation of STAT1 and STAT3 phosphorylation also differed between liver and muscle. For example, in control rats, GH did not significantly increase either pSTAT3 or pSTAT1 in skeletal muscle, which is similar to findings reported previously (8). In contrast, a significant sepsis-induced increase in phosphorylated STAT1 and STAT3 was observed in liver. These results are consistent with other studies indicating that LPS increases STAT1 and STAT3 phosphorylation (34). Additionally, although sepsis increased pSTAT3 in both liver and muscle, the increment in hepatic pSTAT3 was quantitatively greater in magnitude. Although STAT activity is predominantly regulated by posttranslational modifications (i.e., phosphorylation), sepsis also significantly increased the total amount of STAT3 protein in liver and, to a lesser extent, in muscle. This sepsis-induced increase in total STAT3 protein was responsible for the increased STAT3 phosphorylation in muscle, and to a lesser extent, in liver. Increases in total STAT3 have been previously reported in liver of rats in response to thermal injury or LPS (43), and this increase appears to result, at least in part, from an enhanced synthesis of STAT3 (2). Similar changes in the total amount of STAT1 and STAT5 were not observed, which is consistent with previous reports (43).

To investigate potential mechanisms for impaired GH signaling and IGF-I synthesis, we measured the relative content of SOCS and PIAS proteins in muscle and liver. SOCS proteins inhibit intracellular signaling of activated receptor complexes (14, 32, 37). In rat liver, GH induces the expression of SOCS-1, SOCS-2, SOCS-3, and CIS genes, although the kinetics of this induction and the degree of regulation are different for each of these genes (1, 41). Sepsis and GH have also been shown to differentially induce SOCS mRNA in rat liver (37). At present, little is known about the regulation of the SOCS protein in muscle. Recently, Sadowski et al. (35) showed that the mRNA for SOCS-2 and CIS was upregulated by GH in C2C12 muscle cells. However, SOCS-3 protein did not change in this cell line after treatment with GH (16). In the present study, we showed that SOCS-1, SOCS-2, and SOCS-3 proteins were modestly increased in muscle from septic rats. Although sepsis did not alter CIS or PIAS, we cannot exclude the possibility that these proteins were transiently altered at an earlier time point (22). Therefore, at the time GH was injected, septic rats had a mildly elevated content of SOCS-1, -2, and -3 protein in muscle, but this increase was not associated with a reduced ability of GH to stimulate STAT5 phosphorylation. These studies provide the first evidence that sepsis induces SOCS proteins in rat muscle under in vivo conditions.

Previous studies have reported that sepsis increases SOCS-1 and SOCS-3 mRNA content (23). LPS also increases the hepatic expression of SOCS-2, SOCS-3, and CIS mRNA, and these changes are associated with an impairment of STAT5 phosphorylation (9, 30). However, we were unable to detect significant sepsis-induced changes in the content of the SOCS proteins in liver. Consequently, our data do not support their role as important negative regulators of hepatic STAT5 phosphorylation in vivo, as suggested previously (9, 30).

In summary, our results extend observations from previous studies indicating that LPS induces hepatic GH resistance at the level of STAT5 phosphorylation by demonstrating defective hepatic GH signaling in a more clinically relevant model of infection. Importantly, we have shown the inability of GH to increase hepatic IGF-I mRNA content and plasma IGF-I concentrations in septic rats. In contrast to previous studies that measured only SOCS mRNA, we could not detect significant changes in the hepatic content of SOCS proteins, and therefore our data do not support a role for these negative regulators in the development of hepatic GH resistance in sepsis. Additionally, there was a striking disparity between the effect of GH on STAT5 phosphorylation and that on IGF-I mRNA content in skeletal muscle from septic rats. The ability of GH to increase STAT5 phosphorylation was potentiated in muscle of septic rats, and this response was in marked contrast to that of liver. However, when IGF-I mRNA was assessed as a physiological end point of GH stimulation, sepsis prevented the normal increase in muscle IGF-I mRNA observed in control rats. This observation suggests that muscle is indeed “GH resistant,” and that the locus for this defect lies distal to or is independent of STAT5 phosphorylation. The diminished action of GH pertaining to the local synthesis of IGF-I may, in part, be responsible for the altered protein balance in muscle observed in sepsis and other catabolic conditions.

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