Endotoxin attenuates growth hormone-induced hepatic insulin-like growth factor I expression by inhibiting JAK2/STAT5 signal transduction and STAT5b DNA binding

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Endotoxin attenuates growth hormone-induced hepatic insulin-like growth factor I expression by inhibiting JAK2/STAT5 signal transduction and STAT5b DNA binding. Am J Physiol Endocrinol Metab 292: E1856–E1862, 2007. First published February 27, 2007; doi:10.1152/ajpendo.00581.2006.—Gram-negative sepsis with release of endotoxin is a frequent cause of cachexia that develops partly because of resistance to growth hormone (GH) with reduced insulin-like growth factor-I (IGF-I) expression. We set out to more fully characterize the mechanisms for the resistance and to determine whether in addition to a defect in the janus kinase 2 (JAK2)-signal transducer and activator of transcription (STAT) 5b pathway, required for GH-induced IGF-I expression, there might also be a more distal defect. Conscious rats were given endotoxin and studied 4 h later. In liver of these animals, GH-induced JAK2 and STAT5 phosphorylation was impaired and appeared to be caused, at least in part, by a marked increase in hepatic tumor necrosis factor-α and interleukin-6 mRNA expression accompanied by elevated levels of inhibitors of GH signaling, namely cytokine-inducible suppressors of cytokine signaling-1 and -3 and cytokine-inducible SH2 protein (CIS). Nuclear phosphorylated STAT5b levels were significantly depressed to 61% of the control values and represent a potential cause of the reduced GH-induced IGF-I expression. In addition, binding of phosphorylated STAT5b to DNA was reduced to an even greater extent and averaged 17% of the normal control value. This provides a further explanation for the impaired IGF-I gene transcription. Interestingly, when endotoxin-treated rats were treated with GH, there was a marked increase in proinflammatory cytokine gene expression in the liver. If such a response were to occur in humans, this might provide a partial explanation for the adverse effect of GH treatment reported in critically ill patients.

lipopolysaccharide; insulin-like growth factor I; inflammation; cytokines

GRAM-NEGATIVE INFECTIONS with the release of endotoxin induce a catabolic state with increased muscle protein breakdown and decreased protein synthesis. If sustained, this leads to considerable loss of lean body mass, which has an adverse effect on morbidity and mortality (5, 11). Endotoxins are lipopolysaccharides (LPS) derived from the outer bacterial membrane, and its entry in the circulation activates many of the clinical manifestations of sepsis (8). In humans and animals, LPS induces an inflammatory response with the release of glucocorticoids, catecholamines, and proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 that in turn activate the catabolic process (6). This is partly a consequence of a fall in circulating levels, or tissue responsiveness, to anabolic hormones such as growth hormone (GH), insulin-like growth factor I (IGF-I), insulin, and testosterone (6, 34). Administration of LPS causes an early increase in circulating GH and a fall in IGF-I levels in humans, reflecting a GH-resistant state (12). In rodents, resistance to GH also develops, although both the serum GH and IGF-I levels usually fall (20). In addition to this acquired deficiency of IGF-I, tissue resistance to this hormone develops (9), and, together with the increase in inflammatory cytokines and glucocorticoids, leads to a state of accelerated catabolism (6, 11).

The initiating event in GH signaling is binding of hormone to receptor, and this activates janus kinase 2 (JAK2), a protein-tyrosine kinase that in turn phosphorylates the receptor on tyrosine residues (22, 36). These events activate several signaling pathways, including the signal transducer and activator of transcription (STAT) pathway. Activation requires tyrosine phosphorylation of STAT 1a, 3, 5a, and 5b, members of a family of intracellular proteins that serve both as signaling and transcription factors. The phospho-STATs form dimers that enter the nucleus where they bind to specific DNA sequences and activate or repress target genes. Of the STAT proteins, STAT5b plays an essential role in GH-mediated transcription of IGF-I, which is the main mediator of GH action (38, 39) and is the focus of this study. STAT5a, which has 90% sequence homology with STAT5b, may to a lesser extent play a role in mediating GH-stimulated body growth, but it has not been implicated in the regulation of IGF-I gene expression (32). IGF-I is produced throughout the body with liver as the main source of the circulating hormone (39). In cachexic subjects, circulating and local IGF-I levels are reduced, and this deficiency may be compounded by resistance to IGF-I (15, 17). Several different processes regulate the JAK/STAT signaling pathway, including the signal transducers and suppressor of cytokine signaling (SOCS), inhibitory proteins that are induced by GH and other members of the cytokine family (31, 40). Recent studies of LPS- and sepsis-induced inflammation have identified a defect in GH-stimulated JAK2/STAT5 signaling in the liver as a cause of the GH-resistant state and have suggested a role for TNF-α and IL-6 acting in part by inducing expression of the suppressors of cytokine signaling (1, 16, 41).

Because inflammation-induced cachexia is a serious and common problem that arises in part from the development of...
tissue insensitivity to GH, we set out to more fully characterize the mechanism whereby endotoxin induces GH resistance. In particular, we were interested in assessing whether, in addition to a defect in the JAK2/STAT5b pathway, there might also be a more distal defect causing the resistance to GH-induced IGF-I expression. In this study, we confirm that hepatic JAK2-STAT5a/b and nuclear accumulation of STAT5a/b in response to GH is acutely impaired when endotoxin is administered to normal rats. In addition, we found that endotoxin induced a defect in the binding of available nuclear phospho-STAT5b to DNA that likely contributes to the impaired expression of IGF-I. Interestingly, when endotoxin-treated rats were also treated with GH, there was a marked increase in proinflammatory cytokine gene expression in the liver. If such a response were to occur in humans, this might provide a partial explanation for the adverse effect of GH treatment reported in critically ill patients (30).

MATERIALS AND METHODS

Experimental animals and protocols. Male Sprague Dawley rats weighing ~200 grams were studied according to the American Physiology Society guiding principals in the care and use of animals. Inflammation was induced with Escherichia coli-derived LPS (1 mg/kg ip, L4005; Sigma-Aldrich, St. Louis, MO). Control animals were given saline. Later (4 h), the rats were killed, and tissue and blood samples were collected and frozen at −80° and −20°, respectively. The animal protocols were approved by the Institutional Animal Care and Use Committee of the Research Service, Veterans Affairs Palo Alto Health Care System.

GH-stimulated gene expression. To examine the effect of LPS on hepatic GH-stimulated gene expression, rats were treated with LPS or saline, given either recombinant bovine GH (3 mg/kg, a gift from Monsanto, St. Louis, MO) or vehicle by intraperitoneal injection 15 min and again 3 h after the initial injection of LPS or saline, and then killed 1 h later. Eight rats per group were studied.

GH-activated JAK2/STAT5 signal transduction. To examine the effect of LPS on GH-activated signal transduction, 4 h after receiving LPS or saline, rats were anesthetized and via a midline incision, GH (100 µg/kg) or vehicle was injected in the inferior vena cava. Thereafter (15 min), the liver was excised. Six rats per group were studied.

Assay of mRNA levels. Real-time quantitative RT-PCR with SYBR green dye as the detection agent was carried out as before (28) with the ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) using the manufacturer’s protocols (PerkinElmer Applied Biosystems 1997 User Bulletin 2 updated 2001; ABI Prism 7700 Sequence Detection System, Relative Quantitation of Gene Expression, Applied Biosystems). The primers for the assay of the IGF-I, CIS, SOCS1, SOCS2, SOCS3, TNF-α, IL-6 genes, and the internal control gene ribosomal 18S (Table 1) were designed using the primer design software Primer Express (Applied Biosystems) and from published sequences. Total RNA was extracted from liver and used for cDNA synthesis by reverse transcription. The cDNA samples were then subjected to PCR analysis. Results were quantified using the relative standard curve method as described by the supplier. An internal control gene standard curve was also generated, and the target gene was normalized for this endogenous control. Each sample was analyzed in triplicate in individual assays performed on two or more occasions.

Western immunoblotting and immunoprecipitation. The antibodies that detect STAT5a (SC-1081), STAT5b (SC-1656), both STAT5a and STAT5b (SC-835), and protein A and G agarose were from Santa Cruz Biotechnology (Santa Cruz, CA). Phospho-STAT5a/b (05495), JAK2 (06255), and phosphotyrosine-specific (clone 4G10) antibodies were from Upstate Biotechnology (Lake Placid, NY). The GH receptor antibody, directed against the receptor extracellular domain, was a gift from W. R. Baumbach. Liver lysates and nuclear extracts were prepared from frozen liver and used directly for Western immunoblot analysis or subjected to immunoprecipitation before analysis as described previously (25, 29). Tissue lysates (for direct assay of proteins) or immunoprecipitates were heated in Laemmli buffer, separated by electrophoresis on a 7.5% SDS polyacrylamide gel, electroblotted on a nitrocellulose membrane, immunodetected with appropriate antibodies, and visualized by enhanced chemiluminescence. Protein expression was quantified with a Fluor-S digital image analyzer and Multianalyst software (Bio-Rad, Hercules, CA). Relative density units refer to mean pixel density after local background subtraction.

Electrophoretic mobility shift assay. Electrophoretic mobility shift assay (EMSA) was performed as described by Ram et al. (23) with minor modifications as we described before (26). Nuclear extracts were prepared from 100–mg frozen tissue aliquots using the NE-PER nuclear and cytoplasmic extraction reagents (Pierce Biotechnology, Rockford, IL). Nuclear protein (8 µg) dissolved in buffer [10 mM HEPES (pH 7.5), 50 mM NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 0.1 mM EDTA, 1 mM dithiothreitol, 0.5 mM sodium fluoride, 1 mM sodium orthovanadate, 1 µg/ml pepstatin A, and 10% glycerol] was incubated for 10 min at room temperature with 9 µl of gel mobility shift buffer [10 mM Tris–HCl (pH 7.5), 2 µg of poly(dI–dC), 4% glycerol, 1 mM MgCl2, 0.5 mM EDTA, 0.5 mM dithiothreitol, and 50 mM NaCl]. Anti-STAT5b monoclonal antibody was then added and incubated for a further 45 min. Next, the double-stranded oligonucleotide probe, 32P labeled on one strand using T4 polynucleotide kinase, was added and incubated for 30 min. Samples were electrophoresed through non-denaturing polyacrylamide gels (4% acrylamide, 0.05% bisacrylamide). The DNA probe used for the mobility shift analysis was a rat β-casein probe (STAT5b/mammary gland factor response element, nucleotides −101 to −80), 5′-GGA CTT GGA ATT AAG GGA-3′ (sense strand oligonucleotide, ON-101) and 5′-GTC CCT TAA TTC CAA GAA GTT-3′ (antisense strand, ON-257).

Data analysis and statistics. Tyrosine-phosphorylated protein levels were normalized for the respective protein levels. Specific mRNAs were normalized for the internal control gene and are expressed as transcript-to-housekeeping gene ratios. The control vehicle-treated

Table 1. Primer sequences for quantitative real time PCR analysis

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward (5′-3′)</th>
<th>Reverse (5′-3′)</th>
</tr>
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<tbody>
<tr>
<td>IGF-I</td>
<td>GGAATCTCTGTACGATCATATGCTGTG</td>
<td>GGAAGCTTTAGAGGCCAGGCAGCCAAGG</td>
</tr>
</tbody>
</table>
| SOCS1  | TTTGTTGTCGCCGA | TGAATTTTCTTGCGTCTGGTCAGT |}

IGF-I, insulin-like growth factor-I; SOCS, suppressor of cytokine signaling; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.
group mean was given a value of 100, and individual values are expressed relative to this value. Results are given as means ± SE. Two-tailed unpaired Student’s t-tests were used for comparison of two normally distributed groups. Comparisons between more than two normally distributed groups were made by one-way ANOVA followed by pairwise multiple comparison with the Holms t-test (7). A P value <0.05 was considered significant.

RESULTS

IGF-I, cytokine, and SOCS mRNA levels are altered in endotoxemia. In LPS-treated rats, basal IGF-I mRNA levels, measured in vehicle-treated rats, were reduced significantly to 58% of the vehicle-treated control value (Fig. 1). GH administered to controls induced a significant 65% increase in IGF-I mRNA expression; in the LPS-treated rats, the response to GH was suboptimal, and the IGF-I mRNA levels were significantly lower than in the GH-treated controls, reflecting GH resistance. Table 2 summarizes the cytokine and SOCS responses to LPS and GH. LPS alone increased TNF-α mRNA levels 292-fold (P < 0.001), whereas GH alone did not alter the levels. However, when GH was administered to LPS-treated rats, the TNF-α mRNA levels increased even further to 605-fold the basal value (P < 0.001). LPS alone produced a remarkable increase in IL-6 expression to 423-fold the basal control value (P < 0.001), whereas GH alone did not alter the levels. When GH was added to LPS, IL-6 expression increased to 567-fold the basal control value, a value that did not differ statistically from LPS alone.

LPS and GH each caused a significant increase in SOCS mRNA expression when given alone, although the pattern of response differed somewhat (Table 2). LPS increased SOCS1, SOCS3, and CIS mRNA levels by 16-, 10-, and 6-fold, respectively (P < 0.001), but did not affect SOCS2 expression significantly. GH alone increased SOCS2, SOCS3, and CIS mRNA levels 9-, 2-, and 4-fold, respectively (P < 0.001), but did not alter SOCS1 expression. When GH was given to LPS-treated animals, there was a marked further increase in SOCS1 mRNA to 58-fold basal level and a modest increase in SOCS3 mRNA expression; in the LPS-treated rats, the response to GH was suboptimal, and the IGF-I mRNA levels were significantly lower than in the GH-treated controls, reflecting GH resistance.

Table 2. Effect of 4-h of endotoxemia and GH treatment on cytokine and SOCS mRNA levels

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LPS</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>GH</td>
</tr>
<tr>
<td>TNF-α</td>
<td>100±19.1*</td>
<td>78±20.4*</td>
</tr>
<tr>
<td>IL-6</td>
<td>100±48.3*</td>
<td>82±50.2*</td>
</tr>
<tr>
<td>SOCS1</td>
<td>100±19.5*</td>
<td>94.2±12.1*</td>
</tr>
<tr>
<td>SOCS2</td>
<td>100±24.7*</td>
<td>859±209.4‡</td>
</tr>
<tr>
<td>SOCS3</td>
<td>100±17.5*</td>
<td>200±38.6*</td>
</tr>
<tr>
<td>CIS</td>
<td>100±31.4*</td>
<td>406±30.0‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 rats/group. LPS, lipopolysaccharide; GH, growth hormone. Rats treated with LPS or saline (controls) were given GH or vehicle as described in Fig. 1 and killed 4 h after the LPS/saline administration. Hepatic mRNA levels were measured by quantitative real-time PCR and corrected for the internal housekeeping gene 18S rRNA. Results are expressed relative to the control group and assigned a mean value of 100. Dissimilar superscript letters indicate significant difference between groups in each row (P < 0.001). Groups with common superscript letters are similar.

CIS mRNA levels (P < 0.001), but there was no further increase in SOCS3 levels. Interestingly, the SOCS2 response to GH, which is also dependent on STAT5b (39), was significantly depressed in the LPS group. When GH was given to control animals, the SOCS2 mRNA levels increased ninefold, but, when given to LPS-treated rats, it increased only threefold (P > 0.001). Taken together, the cytokine and SOCS responses to LPS observed are consistent with earlier studies that have implicated these proteins as a cause of endotoxin-mediated GH resistance (3, 11, 16). The impact of GH on TNF-α, SOCS1, SOCS2, and CIS mRNA levels in the LPS-treated rats is of special note, since it is conceivable that this response to GH may contribute to the reported adverse effects of GH when administered to endotoxic rats (13, 14) and to critically ill patients (30, 33).

GH-mediated JAK2/STAT5 signal transduction is impaired in endotoxemia. In liver, the GH receptor, JAK2, and STAT5a/b protein levels were similar in the LPS and control groups (Fig. 2A). In animals not given GH, basal tyrosine phosphorylation of STAT5a/b measured in whole liver lysates (Fig. 2B) was significantly reduced in the LPS group compared with the controls (averaging 25 ± 4.5% of the control values, P < 0.001). After a bolus of bovine GH (15 min, 100 µg/kg body wt), tyrosine phosphorylation of JAK2 and STAT5a/b was markedly increased in the control group (Fig. 2C). In the LPS-treated rats, GH-stimulated tyrosine phosphorylation of JAK2 and STAT5a/b in whole liver lysate was depressed significantly (P < 0.01), and the relative values, corrected for protein levels, averaged 51 ± 6.3 and 64 ± 5.4% of the vehicle-injected control values, respectively (Fig. 2D). Nuclear phospho-STAT5a and phospho-STAT5b levels, measured separately (Fig. 3), were also significantly depressed in the GH-treated LPS group and averaged 38 ± 11.8 and 61 ± 5.8% of the control value, respectively (P < 0.01).

STAT5b DNA binding is markedly reduced in acute endotoxemia. The binding of phosphorylated STAT5b to its specific DNA-binding sequence was evaluated in nuclear extracts of animals by EMSA using a 32P-radiolabeled rat β-casein probe and a monoclonal antibody directed against STAT5b only (Fig. 4). GH treatment induced a characteristic gel mobility shift complex, and addition of the STAT5b antibody further retarded the mobility of the band, essentially completely supershifting all DNA-bound radioactivity. LPS reduced the
binding of the β-casein probe to a value, in relative arbitrary densitometry units, that was 17.4 ± 2.5% of the control group value (100 ± 13.3, P < 0.001). This is significantly lower than the reduction in phospho-STAT5b levels in the nucleus (61 ± 5.8% of control value, P < 0.01). This all suggests that, in addition to attenuating phosphorylation and nuclear translocation of STAT5b, LPS has a more distal inhibitory effect on STAT5b binding to DNA, an essential step for inducing IGF-I gene transcription (37, 38).

DISCUSSION

Administration of endotoxin induces a marked systemic response with the release of cytokines, catecholamines, and
glucocorticoids; blood pressure falls; and there are profound metabolic alterations (34, 35). In addition to these systemic perturbations that contribute to the development of GH resistance, it is established that LPS also induces GH resistance by a direct cellular action. This is initiated by the binding of LPS to Toll-like receptors in hepatocytes and muscle cells that mediate the release of proinflammatory cytokines and nitrous oxide that in turn induce a GH-resistant state (6, 21, 24). In this study, we set out to explore the cellular mechanisms whereby endotoxin induces resistance to GH in liver, since this organ is the main source of circulating IGF-I, the levels of which fall when endotoxemia is present (4, 12, 20). Bacterial LPS or saline was administered to normal rats, and they were studied 4 h later. Some rats were treated with GH over this period to stimulate IGF-I gene expression, whereas others received a single bolus of GH to stimulate the JAK2-STAT5 pathway and activate STAT5b binding to DNA, an essential step for initiating IGF-I and also SOCS2 gene expression (39). LPS significantly attenuated basal and GH-stimulated JAK2-STAT5 signaling activity (phosphorylation) and IGF-I gene expression in the liver. This occurred even though the levels of the GH receptor and JAK2, STAT5a, and STAT5b proteins were unchanged. Nuclear translocation of phosphorylated STAT5a and STAT5b was significantly depressed, and phosphorylated STAT5b levels were reduced to 61% of the control values. This represents a potential cause of the reduced IGF-I expression and is consistent with earlier reports of endotoxin- or sepsis-induced hepatic GH resistance (10, 16, 41). In addition, we noted that binding of the phosphorylated STAT5b to DNA was reduced to an even greater extent and averaged 17% of the normal control value. This reduction in DNA-binding activity provides a further explanation for the impaired GH-I gene transcription.

Among the mechanisms likely responsible for the endotoxin-induced inhibition of JAK2-STAT5 signal transduction in the liver is the increase in cytokine and SOCS expression that occurred and that is consistent with earlier reports (3, 41). SOCS proteins are intracellular feedback inhibitors of the JAK-STAT pathway that are induced by members of the cytokine family (31, 40), including GH, which stimulates SOCS-1, -2, and -3 and CIS expression (31, 40). In endotoxin-treated rats, we found that hepatic mRNA levels of TNF-α and IL-6 increased 300- to 400-fold, and this was associated with an increase in SOCS1, SOCS3, and CIS mRNA levels. Of particular interest was the response to GH in endotoxin-treated rats. Although GH did not alter TNF-α or IL-6 expression in the control animals, in the endotoxic rats GH caused a further increase in TNF-α and, to a lesser extent, IL-6 expression; on average, the mRNA levels rose ~600-fold the basal control values. SOCS 1 expression also increased further. In contrast, the SOCS2 response to GH, which is dependent on STAT5b (39), was depressed significantly in endotoxemia. When GH was given to control animals, the SOCS2 mRNA levels increased ninefold, but, when given to endotoxic rats, it increased only threefold. Taken together, it is conceivable that these alterations in the hepatic response to GH may contribute to the GH-induced hypersensitivity to endotoxin-induced injury described in rats (13, 14) and to the increase in morbidity and mortality induced by high-dose GH therapy in critically ill patients (30, 33).

It is well established that inflammation leads to a fall in circulating and local IGF-I levels. In the rat (20), but not in humans (12), this may arise in part because of impaired GH secretion. In addition, some (4), but not all, investigators (16, 41) have reported that GH receptor levels fall in inflammatory states. If this occurs, it would impair GH-mediated signal
transduction. In the present study, GH receptor protein levels were unchanged, but there was a significant defect in GH-mediated JAK2-STAT5 phosphorylation and accumulation of phosphorylated STAT5b in the nucleus. Mao et al. (16) were the first to describe a postreceptor defect in hepatic GH-induced JAK2-STAT5 signaling in the rat that they attributed in part to elevated SOCS3 and CIS levels. This has been confirmed by others (3, 41). Unlike our finding of reduced total phosphorylated JAK2, its level was unchanged in the study of Mao et al. (16). This occurred because of an increase in JAK2 protein levels that compensated for the reduced efficiency of phosphorylation. In addition to the impaired STAT5b phosphorylation and its nuclear accumulation, our observation that endotoxin inhibits the binding of available phospho-STAT5b to DNA provides another cause of the attenuated GH-induced IGF-I and also SOCS2 gene expression. This finding is consistent with Bergad et al. (2) who studied the impact of endotoxin on GH-activated serum protease inhibitor 2.1 gene expression. They noted that STAT5a/b binding to DNA was reduced more than could be accounted for by the reduction in STAT5a/b phosphorylation. Studying septic rats, Yumet et al. (41) reported a decrease in nuclear-phospho-STAT5 levels 18 h after the intraperitoneal inoculation of bacteria; STAT5 DNA-binding activity was also reduced. Because the data were not quantified, it is unclear whether or not the decrease in binding activity was proportional to the fall in phospho-STAT5 levels.

Apart from the contribution of impaired GH-stimulated JAK2-STAT5b phosphorylation and nuclear translocation to the reduced STAT5b DNA-binding activity that we observed in the endotoxic rats, the mechanisms accounting for the disproportional decrease in GH-stimulated STAT5b DNA-binding activity is unclear. Conceivably, this might arise from the action of a protein inhibitor of DNA binding (19) induced by inflammation (2), alterations in the interaction of STAT5b with other transcription factors, or possibly because of altered serine phosphorylation of STAT5b, a process that may modulate STAT5b-binding activity (18). Although these defects can account for impaired GH-stimulated IGF-I gene expression, it is also possible that the attenuated expression may be caused by even more distal events that alter IGF-I mRNA stability or synthesis. Indeed reduced synthesis has been suggested for the inhibitory action of IL-1 on hepatocytes (27). Several recent studies also point to the importance of a defect beyond the JAK2/STAT5 signaling pathway as a cause of impaired GH-induced IGF-I gene expression. Hong-Brown et al. (10), studying skeletal muscle in septic rats, reported that GH-stimulated IGF-I gene expression in these cell types is impaired independent of a defect in STAT5 phosphorylation. Similarly, studies of the inhibitory action of TNF-α and IL-1 in cultured hepatocytes came to the same conclusion (27, 42), and data were presented suggesting that IL-1 inhibited IGF-I mRNA synthesis without affecting stability.

From the present study, we conclude that hepatic resistance to GH-mediated IGF-I expression in acute endotoxemia arises from defects in the postreceptor pathway and beyond. First, activation (phosphorylation) of JAK2 and STAT5 is impaired, and this is caused, at least partly, by proinflammatory cytokines, especially of TNF-α and IL-6, that become overexpressed in liver. In turn, these cytokines increase the levels of SOCS1, SOCS3, and CIS, known inhibitors of GH-induced JAK2-STAT5 phosphorylation. Nuclear accumulation of phosphorylated STAT5b is decreased, and less is available for binding to DNA and initiation of IGF-I gene transcription. Interestingly, these endotoxin-induced abnormalities in JAK2-STAT5 signal transduction are similar to those that we have described in the uremic state and support our postulate that impaired GH-mediated JAK2-STAT5 signal transduction and IGF-I gene expression in uremia may, in part, be caused by inflammation (25, 29). Of note, in addition to the signaling abnormalities, we identified an endotoxin-induced defect in the binding of available nuclear phospho-STAT5b to DNA. Finally, it is worth drawing attention to the cytokine response to GH in endotoxin-treated rats. GH caused a further increase in the endotoxin-elevated proinflammatory cytokine mRNA levels in the liver, and this might conceivably contribute to the adverse of GH reported in critical illness.

GRANTS

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