Burn-induced changes in IGF-I and IGF-binding proteins are partially glucocorticoid dependent

CHARLES H. LANG, GERALD J. NYSTROM, AND ROBERT A. FROST
Departments of Cellular and Molecular Physiology and of Surgery, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

Received 6 June 2001; accepted in final form 19 September 2001

Lang, Charles H., Gerald J. Nystrom, and Robert A. Frost. Burn-induced changes in IGF-I and IGF-binding proteins are partially glucocorticoid dependent. Am J Physiol Regulatory Integrative Comp Physiol 282: R207–R215, 2002; 10.1152/ajpregu.00319.2001.—The purpose of the present study was to determine whether burn-induced changes in various components of the insulin-like growth factor (IGF) system are modulated by the actions of endogenous glucocorticoids or tumor necrosis factor (TNF). To address this aim, a 30% total body surface area full-thickness scald burn was produced in anesthetized rats, and the animals were studied 24 h later. Separate groups of time-matched control and burned rats were pretreated with either an antagonist to glucocorticoids (RU-486) or to TNF (TNF-binding protein; TNFBP). Thermal injury decreased the plasma concentration of IGF-I (38%) as well as the IGF-I mRNA abundance in muscle and kidney (31 and 48%, respectively). While RU-486 prevented the burn-induced decrease in plasma IGF-I, it did not ameliorate the reduction in tissue IGF-I mRNA. Burn increased the plasma concentration of IGF-binding protein (IGFBP)-1 as well as the mRNA content of IGFBP-1 in liver and kidney (15- to 20-fold). These burn-induced increases were partially or largely prevented by RU-486. In contrast, burn decreased the plasma concentration of IGFBP-3 (30%). Burn concomitantly decreased hepatic IGFBP-3 mRNA abundance (42%) but increased IGFBP-3 mRNA in kidney and muscle (50% and 10-fold, respectively). RU-486 largely prevented the burn-induced changes in IGFBP-3 mRNA in kidney and muscle but failed to attenuate the decreases in plasma and liver. Finally, burn injury decreased hepatic acid-labile subunit (ALS) mRNA by 80% and increased the mRNA content of IGFBP-related protein-1 (mac25) in liver by twofold, and these changes were not modified by pretreatment with RU-486. The above-mentioned changes in the IGF system were associated with a burn-induced decrease in muscle protein content that was prevented by RU-486. TNFBP failed to completely ameliorate any of the burn-induced changes in the IGF system. These results demonstrate that glucocorticoids, but not TNF, mediate many but not all of the burn-induced changes in the IGF system.

insulin-like growth factor-binding proteins 1 and 3; acid-labile subunit; mac25; muscle protein; RU-486; tumor necrosis factor-binding protein; rats

ONE OF THE METABOLIC HALLMARKS of burn injury is the prolonged hypermetabolism and the resulting erosion of lean body mass (LBM) (44). Our laboratory and others have previously demonstrated that burn decreases the plasma concentration of insulin-like growth factor (IGF)-I, an important anabolic hormone (1, 26, 28, 35). Furthermore, burn also decreases the IGF-I mRNA and peptide content in skeletal muscle per se (28, 29). This latter finding is particularly relevant because IGF-I has the potential to regulate protein balance and the accretion of LBM via paracrine/autocrine mechanisms (16, 41). In this regard, decreased levels of IGF-I in muscle are positively correlated with decreases in muscle protein synthesis in other catabolic conditions (25, 27).

The bioavailability and bioactivity of IGF-I can also be modulated by changes in one of the IGF-binding proteins (IGFBPs) present in the blood and body fluid compartments (38). Previous studies demonstrate that burn injury decreases the circulating concentration of IGFBP-3 and the acid-labile subunit (ALS) (28). Collectively, these decreases impair the formation of the IGF-I-IGFBP-3-ALS ternary complex that functions as a storage reservoir for IGF-I (38). In contrast, burn injury markedly increases plasma IGFBP-1 concentrations (17, 26, 28). Elevations in IGFBP-1 have been demonstrated to impair IGF-I-mediated increases in glucose uptake and protein synthesis by skeletal muscle (15, 39).

The physiological regulators of the burn-induced changes in the IGF system have not been identified. However, previous studies have demonstrated that tumor necrosis factor (TNF)-α and glucocorticoids play a pivotal role in modulating numerous elements of the IGF system (8, 32) and that their concentration in blood and/or tissues is increased by burn (22, 26, 28, 29). Therefore, the purpose of the present study was to determine whether these two mediators are responsible for the changes in IGF-I and IGFBPs observed in blood and tissues in response to thermal injury.

MATERIALS AND METHODS

Animal preparation and experimental protocol. Adult specific pathogen-free male Sprague-Dawley rats (285–310 g; Charles River Breeding Laboratories, Cambridge, MA) were...
house at a constant temperature, exposed to a 12:12-h light-dark cycle, and maintained on standard rodent chow and water ad libitum for at least 1 wk before experiments were performed. All experiments were approved by the Animal Care and Use Committee at the Pennsylvania State University College of Medicine and adhered to the National Institutes of Health guidelines for the use of experimental animals.

On the day of the experiment, rats were deeply anesthetized with an intramuscular injection of ketamine and xylazine (100 and 10 mg/kg, respectively). The hair on the dorsal and ventral surfaces of the animal was clipped, and animals were secured in an insulated template that exposed only the area of skin to be injured (19). For the burn group, the skin exposed through the template was immersed in 98°C water for 12 s on the dorsal surface and 7 s on the ventral surface. This technique produces a full-thickness scald injury with complete destruction of the underlying neural tissue. The scald injury covered ~30% of the total body surface area (TBSA) and was nonlethal during the 24-h experimental protocol. Rats were dried and then resuscitated with an intraperitoneal injection of lactated Ringer solution (2 ml·kg⁻¹·body surface area burn⁻¹). Control rats were treated the same as those in the burn group, except they were immersed in 25°C water. All rats were injected subcutaneously with the analgesic buprenorphine (0.2 mg/kg) immediately after sham or burn injury. Rats were housed in individual cages and food deprived for the remainder of the experimental protocol. Blood and tissues were sampled ~24 h after the burn because previous studies indicated severe alterations in muscle protein balance and various components of the IGF system at this time (11, 13, 28, 29).

Preliminary studies indicated that plasma TNF-α concentrations determined by ELISA (Biosource, Camarillo, CA) were significantly (P < 0.05) increased at 4 h (68 ± 21 pg/ml) but not at 24 h (below detection limit; <15 pg/ml) compared with values from control rats (<15 pg/ml; n = 5 per group). In contrast, plasma corticosterone concentrations were significantly (P < 0.01) elevated at both 4 and 24 h postburn (414 ± 63 and 372 ± 42 ng/ml, respectively) compared with time-matched control values (241 ± 25 and 143 ± 17 ng/ml, respectively). In addition, we previously demonstrated that the plasma insulin concentration is increased 2.2-fold in burn rats at the 24-h time point compared with values from control rats (28). The hemodynamic status of burn rats was not assessed in the present investigation. Previous studies indicate burn injury produces a transient increase in hematocrit (indicative of fluid extravasation) as well as an early decrease in cardiac output and arterial blood pressure despite standard fluid resuscitation (3, 20, 24). However, by 18–24 h postburn, rats appear hemodynamically stable (3, 24). Therefore, any changes observed in the IGF system are not likely the result of an overt impairment in hemodynamics at the time of death but may have been precipitated by the early transient episode of hypovolemic shock.

To determine the role of endogenously produced TNF-α, separate groups of burn and control rats were injected subcutaneously with TNF-binding protein (TNFBP; 1 mg/kg, 1 ml/rat; Amgen, Boulder, CO), an antagonist of TNF-α action, 4 h before injury. TNFBP is a dimeric, polyethylene glycol-linked form of the human p55 soluble TNF receptor (7). The dose and timing of the synthetic TNF antagonist are based on data demonstrating its ability to prevent the sepsis-induced loss of muscle mass (4). Additional studies were performed to determine the role of glucocorticoids in modulating burn-induced changes in the IGF system. Control and burn rats were injected subcutaneously with saline or the glucocorticoid receptor antagonist RU-486 (20 mg/kg, Mifepristone; Sigma, St. Louis, MO) 30 min before burn or sham injury. RU-486 is an antiprogestin with antiglucocorticoid properties. RU-486 has a high affinity for cytosolic type II glucocorticoid receptors in various target tissues and exhibits little agonist activity (34). The dose of RU-486 used in the present study attenuates glucocorticoid-induced increases in muscle catabolism and ameliorates endotoxin- or cytokine-induced changes in the IGF system (10, 32, 45).

Approximately 24 h after burn injury, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg), and a blood sample (1–2 ml) was collected into heparinized syringes from the abdominal aorta. Selected tissues were excised for the analysis of mRNA content of IGF-I and various IGFBPs. The tissues removed included the gastrocnemius, kidney, and liver (left lateral lobe). Tissues were frozen between liquid nitrogen-cooled aluminum blocks. All plasma and tissue samples were stored at −70°C. A portion of fresh muscle was weighed before and after drying at 110°C to determine dry muscle weight. Muscle protein content was calculated as the product of the weight of the entire gastrocnemius muscle (g) and the muscle protein concentration (mg protein per g dry weight of muscle).

**TNFBP efficacy.** Based on the results from the above-mentioned studies, we believed it was necessary to assess the efficacy of TNFBP to prevent TNF-mediated changes in the IGF system. Therefore, a separate group of rats had a catheter surgically implanted in the jugular vein. The catheter was passed through a tightly coiled stainless steel spring and fixed to a freely rotating swivel (Instech, Plymouth, PA) (8). The infusion assembly permitted the overnight infusion of TNF-α that was begun as soon as the animals regained consciousness. Three groups of rats were included: control rats infused with an equal volume (0.35 ml/h) of vehicle (0.2% BSA in 0.9% saline), rats infused intravenously with a non-dose lethal dose of recombinant human TNF (Amgen, Thousand Oaks, CA, 1 μg·kg⁻¹·h⁻¹), and rats pretreated with TNFBP (1 mg/kg) 4 h before the start of TNF-α. At the conclusion of the 24-h protocol, rats were anesthetized and blood and tissues were collected, as described for the other studies.

**Analytic procedures.** The plasma concentration of total IGF-I was determined using a modified acid-ethanol (0.25 N HCl-87.5% ethanol) procedure with cryoprecipitation followed by radioimmunoassay analysis (8, 25, 28).

Total RNA was isolated using TRI Reagent TR-118 (Molecular Research Center, Cincinnati, OH). Samples (20–100 μg) of total RNA were run under denaturing conditions in 1% agarose-6% formamide gels, and Northern blotting occurred via capillary transfer to Zeta-Probe GT blotting membranes (Bio-Rad Laboratories, Hercules, CA). An 800-bp probe from rat IGF-I (P. Rotwein; St. Louis, MO), a 551-bp probe from mouse IGF-I (M. Kato; Kyoito, Japan) were labeled using a random primed DNA labeling kit (Roche Molecular Biochemicals, Indianapolis, IN). To probe for rat ALS, a 36-mer oligonucleotide was constructed. The complement of the second exon position 3843–3878 (5′-GAC GCT TCG GAG TGC GTT CCT GCT CAG ATC CAG CTC-3′) was the sequence chosen for the oligonucleotide. A rat 18S oligonucleotide was used for normalization of RNA loading. Each oligonucleotide was radiolaactively end-labeled using T4 polynucleotide kinase (Amerham Pharmaica Biotech, Piscataway, NJ). Membranes were hybridized and washed exactly as previously described (28, 29). Membranes were exposed to a phosphorimagery screen, and the resultant data were analyzed using ImageQuant.
software (Molecular Dynamics, Sunnyvale, CA). Data for all probes were normalized to the level of 18S rRNA. Relative mRNA abundance was expressed as the ratio between the particular mRNA and 18S mRNA. This ratio was arbitrarily set at 1.0 for tissues from control (untreated) animals.

IGFBP-1 and ALS content in plasma was determined by Western blot analysis. Plasma samples were separated on 12.5% SDS-PAGE gels under nonreducing conditions (28). Antigen-antibody complexes were identified with goat anti-rabbit IgG tagged with horseradish peroxidase (Sigma) and the enhanced chemiluminescence detection system (Amersham, Arlington Heights, IL). The IGFBP-3 concentration in plasma was determined by Western ligand blot analysis using $^{125}$I-IGF-I, as previously described (8, 25, 28, 32). Samples were subjected to SDS-PAGE without reduction of disulfide bonds. Nitrocellulose sheets were exposed to X-ray film (Kodak X-OMAT AR; Eastman Kodak, Rochester, NY) and intensifying screens (DuPont, Wilmington, DE). The resulting bands were scanned (Microtek ScanMaker IV) and analyzed using NIH Image 1.6 software. Samples from all experimental groups were electrophoresed on the same gel; data are expressed as a percentage of the control value.

Statistics. Data were obtained from three separate experimental series each containing control and burn rats. For each study, rats were randomly assigned to either the control or experimental group. Values are presented as means ± SE. The number of rats per group is indicated in the legends to Figs. 1–9. Data were analyzed by ANOVA followed by Student-Newman-Keuls to determine treatment effect. When heterogeneity of variance was detected, data were transposed using Newman-Keuls to determine treatment effect. When experimental groups were electrophoresed on the same gel; values are presented as means ± SE for the densitometric analyses of the blots are presented in RESULTS.

RESULTS

Plasma concentrations of IGF system components. Twenty-four hours after thermal injury, the circulating concentration of IGF-I was decreased 38% (Fig. 1). Pretreating rats with the glucocorticoid receptor antagonist RU-486 completely prevented the burn-induced decrease in IGF-I. In contrast, treatment of rats with TNFBP was largely ineffective at maintaining the plasma IGF-I concentration.

Figure 2, top, illustrates that, compared with control values (32 ± 5 AU), plasma IGFBP-1 was increased 19-fold after burn (598 ± 77 AU; $P < 0.05$). This increase was not significantly attenuated by TNFBP (417 ± 68 AU) but largely prevented by RU-486 (121 ± 25 AU; $P < 0.05$ compared with burn and control values). Ligand blot analysis demonstrated an ~35% decrease in IGFBP-3 levels in burn rats (256 ± 31 AU) compared with values from control animals (348 ± 27 AU; $P < 0.05$ compared with burn values), and this reduction was not significantly altered by either TNFBP or RU-486 (211 ± 32 and 253 ± 27 AU, respectively) (Fig. 2, middle). Similarly, burn also decreased the plasma concentration of ALS by ~30% (control = 531 ± 29 AU vs. burn = 377 ± 41 AU; $P < 0.05$), and this decrease was still present in rats pretreated with either TNFBP or RU-486 (359 ± 42 and 388 ± 47 AU, respectively) (Fig. 2, bottom).

Tissue IGF-I and IGFBP mRNA abundance. Northern blot analysis revealed that 24 h after thermal injury, there was an ~50% increase in the 7.5-kb IGF-I mRNA transcript in liver (Fig. 3). Other IGF-I mRNA transcripts of lesser abundance (e.g., 1.7 kb and 0.9–1.2 kb) demonstrated qualitatively similar changes (data not shown). RU-486 prevented the burn-induced increase in hepatic IGF-I mRNA, while TNFBP partially ameliorated the response. In contrast to liver, muscle (gastrocnemius) and whole kidney demonstrated a significant reduction in IGF-I mRNA after burn (31 and 48%, respectively). The reduction in both tissues was unaffected by pretreatment with either TNFBP or RU-486.

The abundance of IGF-II mRNA was also determined in gastrocnemius but was not altered by thermal injury (control = 1.0 ± 0.07 AU vs. burn = 1.05 ± 0.11 AU; $P = \text{NS}$).
IGFBP-1 mRNA abundance was increased 15-fold in liver and 25-fold in kidney (Fig. 4). Administration of RU-486 reduced the burn-induced increase in IGFBP-1 mRNA content by 45–65% in both tissues, whereas pretreatment with TNFBP resulted in no statistically significant changes. IGFBP-1 mRNA was not detected in muscle by Northern blot analysis.

Hepatic IGFBP-3 mRNA content was decreased 42% after burn injury, and this reduction was not prevented by either TNFBP or RU-486 (Fig. 5). In contradistinction to liver, burn increased IGFBP-3 mRNA in kidney (53%) and skeletal muscle (~10-fold). RU-486 completely prevented the burn-induced increase in IGFBP-3 mRNA in kidney and reduced the increase in muscle by ~85%. The TNF antagonist partially ameliorated the burn-induced increase in IGFBP-3 mRNA in kidney but did not attenuate the increase observed in muscle.

Thermal injury decreased ALS mRNA abundance in liver by ~80%, and neither TNFBP nor RU-486 pre-
vented this decline (Fig. 6). No burn- or drug-induced changes in ALS mRNA were observed in kidney (data not shown), and ALS mRNA was not detected in muscle.

The expression of IGFBP-related protein-1 (IGFBP-rP1), also termed mac25 or IGFBP-7, was increased twofold in liver in response to thermal injury (Fig. 7). Neither RU-486 nor TNFBP prevented the burn-induced increase in IGFBP-rP1. No burn- or drug-induced changes in IGFBP-rP1 were observed in either skeletal muscle or kidney (data not shown).

**Muscle protein content.** Because changes in the IGF system may impact on muscle protein balance, the protein content (mg protein/muscle) of the gastrocnemius muscle was assessed. Muscle protein content was significantly decreased 8.5% 24 h after thermal injury (Fig. 8). Whereas a similar decrement in protein content was seen in burn rats administered TNFBP, pretreatment with RU-486 completely prevented the loss of muscle protein.

**Efficacy of TNFBP.** As described above, the dose of TNFBP used in this study was selected based on its reported ability to prevent the decrease in muscle protein synthesis induced by bacterial infection (4). However, based on the generalized inability of this drug to antagonize the burn-induced changes in the IGF system in the current study, we performed a limited “positive-control” study designed to verify the efficacy of TNF blockade on a restricted number of IGF system components. Figure 9 shows that infusion of TNF-α for 24 h into naive control animals decreased the plasma IGF-I concentration, decreased the muscle content of IGF-I mRNA, and increased the hepatic abundance of IGFBP-1 mRNA. Additionally, these data indicate that the same dosing regimen that failed to prevent IGF changes in burned rats was effective at preventing the changes induced by the TNF-α infusion. Therefore, it appears that the failure of TNFBP to prevent burn-induced changes in the IGF system is not the result of insufficient drug availability or bioactivity.

**DISCUSSION**

Alterations in various components of the IGF system are a well-recognized characteristic of the hormonal response to thermal injury (14); however, the etiology of these changes has not been elucidated. Burn has been reported either to increase the circulating concen-
tation of TNF-α or to increase the tissue expression of this cytokine (2, 20). Our preliminary data confirm a transient increase in the plasma TNF-α concentration after burn. Previous studies have also demonstrated that intravenous infusion of TNF-α into naive control animals produces many alterations that are comparable to those observed after burn injury (Refs. 8 and 9 and present data). Moreover, antagonizing the actions of TNF with a neutralizing antibody prevents or clearly attenuates many of the changes in IGF-I and IGFBPs produced in response to intravenously administered E. coli endotoxin or zymosan-induced peritonitis (8, 9). Collectively, these findings formed the scientific rationale for treating animals with TNFBP in the present study. Our data indicate that TNFBP was unable to completely prevent any of the burn-induced changes in the IGF system. TNFBP did modestly attenuate the decrease in plasma IGF-I and the increase in plasma IGFBP-3 mRNA abundance. However, the lack of consistent and striking changes between TNFBP-treated and vehicle-treated burn rats suggests that endogenously produced TNF-α is a relatively minor modulator of the IGF system after thermal injury. Furthermore, TNFBP was also unable to prevent the burn-induced decrease in muscle protein content, suggesting that endogenous TNF-α is not a primary mediator for the observed muscle atrophy. This response differs considerably from that seen in septic rats, where the decrease in muscle protein synthesis and muscle wasting was prevented by chemical inhibitors of TNF-α production (23). Taken together, these data imply that thermal injury and sepsis produce changes in the IGF system and muscle protein balance by different mechanisms.

Fig. 6. Effect of TNFBP and RU-486 on burn-induced changes in hepatic mRNA abundance of the ALS. Values are means ± SE; n = 14, 12, 6, 8, 7, and 8 (for groups designated by bars, left to right). Time-matched control (open bars) and burn (solid bars) rats were studied under control conditions (basal) or after pretreatment with either TNFBP or RU-486. Tissue was collected 24 h after injury and analyzed as described in MATERIALS AND METHODS. Inset: representative Northern blot for ALS from the 6 different treatment groups. For bar graph, hepatic ALS mRNA abundance is expressed in AU determined by phosphorimaging and normalized to 18S rRNA; 18S rRNA indicated that the amount of RNA loaded was similar for each lane (data not shown). Groups with different letters are significantly different from each other (P < 0.05).

Fig. 7. Effect of TNFBP and RU-486 on burn-induced changes in hepatic mRNA abundance for IGFBP-related protein-1 (IGFBP-rP1). Values are means ± SE; n = 14, 12, 6, 8, 7, and 8 (for groups designated by bars, left to right). Time-matched control (open bars) and burn (solid bars) rats were studied under control conditions (basal) or after pretreatment with either TNFBP or RU-486. Tissue was collected 24 h after injury and analyzed as described in MATERIALS AND METHODS. Inset: representative Northern blot for IGFBP-rP1 in liver from the 6 different treatment groups. For bar graph, hepatic IGFBP-rP1 mRNA abundance is expressed in AU determined by phosphorimaging and normalized to 18S rRNA; 18S rRNA indicated that the amount of RNA loaded was similar for each lane (data not shown). Groups with different letters are significantly different from each other (P < 0.05).

Fig. 8. Effect of TNFBP and RU-486 on burn-induced changes in muscle protein content. The protein content of the gastrocnemius muscle was calculated as the product of the whole muscle weight (g) and the muscle protein concentration (mg protein/g dry weight of muscle). Values are means ± SE; n = 14, 12, 6, 8, 7, and 8 (for groups designated by bars, left to right). Time-matched control (open bars) and burn (solid bars) rats were studied under control conditions or after pretreatment with either TNFBP or RU-486. Tissue was collected 24 h after injury and analyzed as described in MATERIALS AND METHODS. Groups with different letters are significantly different from each other (P < 0.05).
Abundance in liver (bottom), and IGFBP-1 mRNA abundance in gastrocnemius (middle) were examined included the plasma IGF-I concentration (top), IGF-I/H9251 treated with TNF-α (hatched bars) or TNFBP (1 mg/kg; solid bars) 4 h before the start of infusion. Representative elements of the IGF system that were examined included the plasma IGF-I concentration (top), IGF-I mRNA abundance in gastrocnemius (middle), and IGFBP-1 mRNA abundance in liver (bottom). The IGF-I mRNA and IGFBP-1 mRNA content were expressed in AU determined by phosphorimaging and normalized to 18S rRNA; 18S rRNA indicated that the amount of RNA loaded was similar for each lane (data not shown). Values are means ± SE; n = 5, 5, and 6 (for groups designated by bars, left to right). Groups with different letters are significantly different from each other (P < 0.05).

Fig. 9. Efficacy of TNFBP in preventing TNF-α-induced changes in the IGF system. Rats had a venous catheter surgically implanted and were connected to a swivel apparatus for the continuous infusion of a nonlethal dose of recombinant human TNF-α (1 μg/kg·h) or 0.2% BSA (time-matched controls; open bars) for 24 h. TNF-α-infused rats were injected subcutaneously with either saline (hatched bars) or TNFBP (1 mg/kg; solid bars) 4 h before the start of the TNF-α infusion. Representative elements of the IGF system that were examined included the plasma IGF-I concentration (top), IGF-I mRNA abundance in gastrocnemius (middle), and IGFBP-1 mRNA abundance in liver (bottom). The IGF-I mRNA and IGFBP-1 mRNA content were expressed in AU determined by phosphorimaging and normalized to 18S rRNA; 18S rRNA indicated that the amount of RNA loaded was similar for each lane (data not shown). Values are means ± SE; n = 5, 5, and 6 (for groups designated by bars, left to right). Groups with different letters are significantly different from each other (P < 0.05).

cortisol in humans (26, 28, 29). Furthermore, glucocorticoids are known to alter numerous elements of the IGF system (31, 33, 42). It is noteworthy that injection of RU-486 before burn prevented the reduction in the plasma concentration of IGF-I, and this change was associated with the maintenance of muscle protein content. These data complement previous studies demonstrating that exogenous administration of IGF-I to rats reversed burn-induced changes in muscle protein synthesis and degradation (12) and that administration of an IGF-I/IGFBP-3 complex to severely burned humans improves leg muscle protein balance (5). Other types of catabolic illness are also characterized by a decrease in circulating IGF-I that is temporally associated with a reduction in hepatic IGF-I mRNA (8, 10, 25, 27). However, in the present study, hepatic IGF-I mRNA abundance actually increased after thermal injury. Previously, we demonstrated that despite the increase in IGF-I mRNA, there was a corresponding decrease in the IGF-I peptide content of liver from burned rats (28). This dichotomy suggests that in the liver, glucocorticoids downregulate the translation of IGF-I mRNA or that the stability of the IGF-I peptide is decreased during glucocorticoid excess. In contrast to blood and liver, RU-486 was unable to prevent the burn-induced decrease in IGF-I mRNA content in either muscle or kidney.

An increase in circulating IGFBP-1 is observed in many catabolic and inflammatory states, including human immunodeficiency virus infection, alcohol abuse, sepsis, endotoxemia, and cytokine administration (8, 10, 14, 25, 27). Elevations in IGFBP-1 impair whole body glucose disposal and protein synthesis in myocytes (15, 39), and therefore the physiological regulators of this particular binding protein are of considerable clinical relevance. The liver and kidney are the two organs in male rats that have the highest mRNA abundance for IGFBP-1 (30). In this regard, IGFBP-1 mRNA content was increased 15- to 25-fold in these two organs after burn injury. The burn-induced increase in IGFBP-1 appears to be largely, but not completely, mediated by glucocorticoids. Our data demonstrate that RU-486 dramatically attenuates the increase in plasma IGFBP-1 in burned rats, and this change was associated with a concomitant reduction in the abundance of IGFBP-1 mRNA in both liver and kidney. Similarly, increases in circulating IGFBP-1 induced by hypoglycemia or diabetes also appear to have a glucocorticoid-dependent component (31, 42). These data are consistent with previous studies in which in vivo injection of dexamethasone increased both hepatic synthesis and the plasma concentration of IGFBP-1 (33). Because RU-486 failed to completely prevent the burn-induced increases in either plasma or tissue IGFBP-1, other factor(s) must also be involved. Under nonstress conditions, the plasma concentration and hepatic synthesis of IGFBP-1 are inversely proportional to the prevailing plasma insulin concentration (30). However, this mechanism does not appear operational in burned rats because plasma insulin concentrations are reported to be slightly increased in response to thermal injury (28).

The majority of IGF-I in the blood is carried as part of a ternary complex, consisting of the ligand, IGFBP-3, and ALS (39). Because of its large molecular weight (150 kDa), the complex is compartmentalized to blood and may function as a reservoir for IGF-I. We previously demonstrated that burn decreases the plasma IGFBP-3 concentration in rats and humans (26, 28). The liver is a major contributor to the bloodborne IGFBP-3, but other tissues may also be important in the homeostasis of this binding protein (39). Although IGFBP-3 synthesis is considered growth hor-
mone (GH) dependent, relatively little is known regarding its regulation during stress conditions. In the present study, burn injury produced discordant changes in tissue IGFBP-3 mRNA abundance. Hepatic IGFBP-3 mRNA content was clearly reduced by burn, whereas expression was moderately increased in kidney (−50%) and markedly increased in skeletal muscle (−10-fold). Furthermore, the burn-induced increase in IGFBP-3 mRNA in the latter two tissues was largely prevented by RU-486, while this drug failed to ameliorate the decrease in hepatic IGFBP-3 mRNA content. These data indicate a tissue-specific regulation of IGFBP-3 synthesis by an as yet unknown mechanism and support the concept that liver is the primary site of production of circulating IGFBP-3.

Formation of the ternary complex is dependent on adequate synthesis of ALS. Burn injury markedly decreased both the plasma concentration and hepatic mRNA content for ALS. The amount of ALS in the blood is primarily determined by GH-dependent transcriptional activation of the ALS gene within the liver (37). As such, decreases in the plasma GH concentration or the presence of GH resistance are likely mediators (6). Ogle et al. (36) recently reported that thermal injury increases the hepatic content of suppressors of cytokine signaling (SOCS)-3, which has been reported to antagonize GH action in this tissue. The presence of hepatic GH resistance would also be consistent with the burn-induced decrease in hepatic IGFBP-3 mRNA. Pretreatment with RU-486 failed to prevent the burn-induced decrease in ALS in plasma and liver, and hence this response like that of IGFBP-3 in blood and liver appears to be relatively independent of the actions of glucocorticoids. Finally, ALS mRNA content in kidney was not altered by burn. These data suggest that renal and hepatic ALS synthesis may be regulated differently and that the burn-induced decrease in ALS is primarily the result of changes occurring within the liver.

There are several newly discovered proteins that have a relatively low sequence homology and a reduced binding affinity for IGF-I compared with the six traditional IGFBPs (21). In the present study, we examined whether burn alters the expression of one of these IGFBP-related peptides, IGFBP-rP1. This particular IGFBP-rP was of interest because it has been reported to impair insulin binding to its cognate receptor and decrease tyrosine phosphorylation of the insulin receptor and insulin-receptor substrate 1 (43), and therefore it may be a potential mediator of the peripheral insulin resistance observed in response to burn injury (40). Of the limited number of tissues examined, only liver demonstrated an increase in IGFBP-rP1 mRNA abundance 24 h after burn. It is unknown whether increases in hepatic IGFBP-rP1 mRNA are indicative of increased rates of synthesis for this protein and whether a concomitant increase in circulating levels of IGFBP-rP1 might be expected. Changes in IGFBP-rP1 in other catabolic states have not been reported. There are no data in the literature pertaining to the in vivo regulation of this binding protein, and our data suggest that neither TNF-α nor glucocorticoids are important regulators of the burn-induced increase in hepatic IGFBP-rP1 mRNA content or to the constitutive basal expression in any of the tissues examined.

The above-mentioned changes in the IGF system were associated with a decrease in gastrocnemius protein content that was prevented by pretreatment with RU-486. This finding is consistent with a previous study demonstrating that RU-486 attenuated the burn-induced decrease in muscle weight primarily by preventing the increased rate of muscle protein degradation (18). Again, the mechanisms responsible for the muscle wasting produced by burn and sepsis appear to differ because RU-486 was unable to attenuate the enhanced rate of proteolysis observed in the latter condition (11). Our data indicate that many, but not all, of the burn-induced changes in the IGF system are also glucocorticoid dependent and TNF independent. The ability of RU-486 to prevent or greatly attenuate the decrease in plasma IGF-I, the increase in IGFBP-1 in the blood and muscle, and the increase in IGFBP-3 within muscle may represent potential mechanisms helping to limit the burn-induced erosion of LBM. Finally, these data suggest that the relative importance of glucocorticoids and TNF-α in regulating these different components of the IGF system may also vary depending on the specific catabolic condition.

We thank X. Liu for excellent technical assistance and express sincere thanks to all the investigators who provided the cDNA probes used in this study: Drs. P. Rotwein (IGF-I), H. Whitfield (IGF-II), S. Shimisaki (IGFBP-1 and IGFBP-3), and M. Kato (mac25).

We thank the National Hormone and Pituitary Program (National Institute of Diabetes and Digestive and Kidney Diseases) for the generous gift of the insulin-like growth factor (IGF)-I antibody, and we thank Genentech (South San Francisco, CA) for the IGF-I used in these studies. We extend sincere gratitude to Amgen (Boulder, CO) for generously providing the tumor necrosis factor (TNF)-binding protein and the human recombinant TNF-α used in this study.

This work was supported in part by National Institutes of Health Grants GM-38032 and HL-66443.

REFERENCES


