Regulation of myostatin by glucocorticoids after thermal injury

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SPECIFIC AIM
The purpose of the present study was to test the hypothesis that the catabolic state induced by burn injury, sepsis, or endotoxemia is associated with an increase in muscle myostatin mRNA content and a concomitant reduction in muscle IGF-I or IGF-II mRNA. The role of endogenous glucocorticoids and tumor necrosis factor α (TNF-α) in mediating the burn-induced changes in myostatin mRNA was also assessed by treating rats with selective antagonists to these catabolic factors.

PRINCIPAL FINDINGS
1. Effect of burn, endotoxin, or sepsis on myostatin mRNA
A full-thickness scald injury covering ~30% of the total body surface area was produced in male rats. Twenty-four hours after thermal injury, Northern blot analysis indicated that steady-state myostatin mRNA levels were increased more than threefold in the gastrocnemius (C=1.0±0.2 AU vs. B=3.3±0.4 AU; P < 0.05). In contrast, the injection of Escherichia coli endotoxin (100 μg/100 g BW) or the induction of a polymicrobial sepsis did not significantly alter myostatin mRNA vs. appropriate time-matched control animals. Myostatin mRNA was not detected in other muscles (soleus or heart) or nonmuscle tissues (liver and kidney) in either control or stressed rats using Northern blot analysis. Twenty-four hours after injury, the decrease in muscle protein content (in mg protein per gram dry weight) was comparable in response to the different traumatic insults (burn=108±3* vs. control 117±2; LPS=98±4* vs. control=113±3; sepsis=103±4* vs. control=114±2; *P<0.05 vs. appropriate control value).

2. Effect of injury on tissue IGF-I mRNA and plasma corticosterone
Thermal injury decreased the abundance of IGF-I mRNA by 45% in gastrocnemius. Endotoxin decreased muscle IGF-I mRNA by 58% at 4 h and 32% at 24 h. Finally, IGF-I mRNA was reduced 29% in gastrocnemius from septic rats. In contrast, none of the traumatic injuries significantly altered IGF-II mRNA levels in gastrocnemius. The plasma corticosterone concentration was increased after burn injury (141%) compared with control values. Corticosterone was also increased 4 h after endotoxin administration (104%), but levels had returned to control values by 24 h. A small, albeit statistically significant, 25% increase in corticosterone was detected in rats 24 h after induction of sepsis.

3. Endogenous glucocorticoids mediate burn-induced increase in myostatin
To address the role that endogenous glucocorticoids play in modulating the burn-induced increase in myostatin, rats were pretreated with the glucocorticoid receptor antagonist RU486 (20 mg/kg). Figure 1 (top panel) illustrates that burn increased myostatin mRNA by ~fourfold and this elevation was almost completely prevented in rats administered RU486. RU486 also prevented the burn-induced reduction in gastrocnemius protein content (Fig. 2, bottom panel). Neither burn injury nor RU486 significantly altered the protein content of the soleus muscle (data not shown).

4. Dexamethasone increases myostatin mRNA
Based on the above-mentioned results, we speculated that the exogenous administration of glucocorticoids to naive control animals should also be able to elevate myostatin mRNA. Figure 2 (top panel) illustrates that myostatin mRNA was increased 60% and 2.7-fold in gastrocnemius at 4 h and 24 h, respectively, after a single injection of dexamethasone (100 μg/100 g BW). Figure 2 (bottom panel) also illustrates that pretreatment with RU486 completely prevented the dexamethasone-induced increase in myostatin, demonstrating the efficacy of the glucocorticoid antagonist.

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5. Role of endogenous TNF-α in mediating burn-induced increase in myostatin

Several studies have demonstrated that burn injury is associated with an elevation in plasma or tissue levels of TNF-α and that administration of TNF-α to control animals stimulates loss of muscle protein. Hence, additional rats were treated with TNF binding protein (TNFBP; 1 mg/kg, Amgen, Thousand Oaks, CA) before thermal injury to antagonize the in vivo effects of this cytokine. TNF BP failed to significantly prevent either the burn-induced increase in myostatin mRNA in gastrocnemius or the reduction in muscle protein content.

CONCLUSIONS AND SIGNIFICANCE

Natural mutations in the myostatin gene in several breeds of cattle and experimental deletion of the gene in mice dramatically increase skeletal muscle mass. Conversely, other studies indicate an inverse correlation between myostatin-immunoreactive protein in serum and muscle and lean body mass in HIV-infected patients with weight loss. Alterations in myostatin expression have not been assessed in other catabolic conditions characterized by the erosion of lean body mass. In the present study, the burn-induced increase in myostatin was associated with a reduction in the protein content of the gastrocnemius muscle. Previous studies have demonstrated that the burn-induced alterations in protein metabolism are more pronounced in...
Burn-Induced Increase in Muscle Myostatin

**Figure 3.** Schematic representation of the mechanism involved in regulating myostatin mRNA expression in response to burn. Thermal injury activates a number of stress-response systems. Stimulation of the hypothalamic-pituitary-adrenal (HPA) axis results in the release of glucocorticoids and various macrophage/monocytes-type cells release of various proinflammatory cytokines, including TNF-α. The increase in muscle myostatin mRNA observed after burn appears to be dependent on the increase in glucocorticoids but independent of the rise in TNF-α.

fast-twitch vs. slow-twitch skeletal muscle, and this differential response is consistent with the observed increase in myostatin in the gastrocnemius but not the soleus. Moreover, these changes in protein metabolism and myostatin occurred in a muscle distant from the site of thermal injury, suggesting the involvement of one or more neurohumoral agents. The increase in myostatin, however, did not appear to be a generalized response to catabolic stimuli. No increase in myostatin mRNA was detected at a similar time after injection of endotoxin or induction of peritonitis despite a comparably decreased muscle protein content.

The three catabolic insults appear to elicit a glucocorticoid response that differs in magnitude and/or duration. Burn injury results in a rapid and sustained elevation in circulating corticosterone. Endotoxin also increased plasma corticosterone concentrations to a comparable level at 4 h, but concentrations had returned to basal values by 24 h. In rats with peritonitis, the plasma corticosterone concentrations were only mildly elevated when the animals were killed. Hence, these data suggested that a sustained, large elevation in glucocorticoids might be responsible for the burn-induced increase in myostatin. This conclusion is supported by published data indicating that injection of a synthetic glucocorticoid impairs muscle protein balance and that pretreatment with the RU486 is able to prevent the burn-induced increase in muscle protein degradation and attenuate the loss of muscle protein.

We investigated the regulatory role of glucocorticoids by determining the myostatin response in control animals injected with dexamethasone. Animals so treated demonstrated increased levels of myostatin mRNA comparable to that observed in burn rats. In the complementary study, RU486 was administered before burn injury and prevented both the increase in myostatin and the decrease in muscle protein content. Collectively, these data indicate that sustained elevations in circulating glucocorticoids are able to increase myostatin and that an elevation in the endogenous levels of this hormone is largely responsible for the increased myostatin observed in response to thermal injury.

Burn also increases the TNF-α concentration in blood or tissue and appears to be an important regulator of the muscle wasting accompanying sepsis. However, the TNF antagonist TNFβP was unable to significantly prevent either the burn-induced increase in myostatin or the decrease in muscle protein content. These data suggest that overexpression of TNF-α does not play a major role in regulating myostatin expression during this particular traumatic condition.

IGF-I and IGF-II are critical for the normal development and maintenance of lean body mass. An inverse relationship has been reported for these ligands and the abundance of myostatin in response to muscle atrophy or regeneration. However, in the current investigation, neither burn, endotoxin, nor sepsis significantly altered IGF-II mRNA content in gastrocnemius. In contrast, all three catabolic insults produced a comparable decrease in muscle IGF-I mRNA. These latter data support the hypothesis that trauma-induced changes in IGF-I may be an important regulator of muscle mass but fail to support a strong association between changes in IGF-I and myostatin.

In summary, these data indicate that myostatin mRNA levels can be increased in adult rats by exogenous glucocorticoids and that the endogenous elevation of corticosterone is a major regulator of the increased myostatin and decreased muscle protein content observed after thermal injury. In contrast, our results do not support a regulatory role for TNF-α, IGF-I, or IGF-II in the in vivo control of myostatin mRNA abundance.