The purpose of this study was to test the hypothesis that muscle proteolysis induced by TNF or IL-1 is mediated by glucocorticoids. Rats were treated with 300 μg kg⁻¹ of recombinant human preparations of IL-1α (rIL-1α) or TNFα (rTNFα) divided into three equal intraperitoneal doses given over 16 h. Two hours before each cytokine injection, rats were given 5 mg kg⁻¹ of the glucocorticoid receptor blocker mifepristone RU 38486, by gavage or were gavaged with the vehicle. Eighteen hours after the first cytokine injection, total and myofibrillar protein breakdown rates were determined in incubated extensor digitorum longus muscles as release of tyrosine and 3-methylhistidine, respectively. Total and myofibrillar proteolytic rates were increased following injection of rIL-1α or rTNFα. Proteolysis induced by rIL-1α was not altered by treatment with RU 38486. In contrast, the glucocorticoid receptor blocker inhibited the proteolytic effect of rTNFα. The results suggest that the proteolytic effect of TNF is mediated by glucocorticoids and that IL-1 induces muscle proteolysis through a glucocorticoid independent pathway.

Keywords: Glucocorticoids; IL-1; Protein breakdown; Skeletal muscle; TNF.

Introduction

Previous studies suggest that tumour necrosis factor (TNF) and interleukin-1 (IL-1) can stimulate muscle protein breakdown, although conflicting results have been reported. The mechanism by which TNF and IL-1 exert this metabolic effect is unknown. A direct effect on protein metabolism could not be verified when IL-1 or TNF was added to incubated muscles. Because both cytokines can induce release of glucocorticoids it has been suggested that the metabolic effects of these cytokines involve hormonal stimulation. The purpose of this study was to test the hypothesis that muscle proteolysis induced by TNF or IL-1 is mediated by glucocorticoids. This was done by administering the cytokines to rats that had been treated with the glucocorticoid receptor antagonist RU 38486.

Materials and Methods

The study was performed using male Sprague-Dawley rats weighing 40–60 g (Zivic-Miller Laboratories, Inc., Zelienople, PA). The animals were housed in groups of three per cage at 22°C room temperature with a 12 h light/dark cycle, and were allowed to acclimatize for 3 days before experiments. Three series of experiments were performed. In the first series of experiments, groups of rats were treated with three intraperitoneal injections of human recombinant IL-1α (rIL-1α), 100 μg kg⁻¹ body weight each, at 16:00, 24:00 and 08:00 h. The cytokine (kindly provided by Hoffman-La Roche, Nutley, NJ) had a specific activity of 3 x 10⁸ U mg⁻¹ protein (D10 assay) and contained less than 0.75 endotoxin units/mg protein (determined by Limulus lysate assay). The cytokine was diluted in phosphate buffered saline (PBS), pH 7.4, to a final concentration of 10 μg ml⁻¹. Other rats received corresponding intraperitoneal injections of PBS.

In the second series of experiments, an identical protocol was employed except that human recombinant TNFα (rTNFα) was injected instead of rIL-1α. The rTNFα preparation (kindly provided by Knoll Pharmaceuticals, Whippany, NJ) had a specific activity of greater than 5 x 10⁶ U mg⁻¹ protein (L929 cytotoxicity test) with an endotoxin content of less than 5 U mg⁻¹ protein (Limulus lysate assay).
Muscle protein breakdown rates were measured 2 h after the last cytokine or PBS injection. With rats under ether anaesthesia, the extensor digitorum longus muscles were dissected with their tendons intact. The muscles were tied by their tendons on stainless steel supports at resting length and incubated individually in 3 ml of oxygenated (O₂:CO₂, 95:5) Krebs-Henseleit bicarbonate buffer (pH 7.4) with 10 mM glucose in a shaking water bath at 37°C. After preincubation for 30 min, the muscles were incubated for 2 h in 3 ml of fresh oxygenated medium of the same composition as described above but also containing cycloheximide (0.5 mM) to prevent reutilization of amino acids released during proteolysis. Total and myofibrillar protein breakdown rates were determined as release of tyrosine and 3-methylhistidine (3-MH), respectively, taking changes in tissue levels of the amino acids during incubation into account, as described in detail previously.14

In the third series of experiments, we examined the effect of rIL-1α and rTNFα on plasma levels of corticosterone, the predominant glucocorticoid in rodents. rIL-1α or rTNFα was injected intraperitoneally at a dose of 100 μg kg⁻¹ body weight at 08:00 h. Control animals received corresponding volumes of PBS. Blood was collected into heparinized beakers following decapitation 30 min, 2 h, 4 h and 8 h after cytokine or PBS injection. Blood was centrifuged and the plasma was stored at −70°C until assayed. Corticosterone levels were determined by radioimmunoassay.15

Results are presented as mean ± SEM. For statistical comparison analysis of variance followed by Duncan’s multiple range test was used. Differences were considered significant for p < 0.05.

Results

Rats exhibited symptoms of lethargy, piloerection and loose stool following injection of rIL-1α or rTNFα, and these symptoms were slightly more prominent following rIL-1α than rTNFα administration. There was no mortality when rIL-1α or rTNFα was injected into rats that had not been treated with RU 38486. In the groups of rats that had been treated with RU 38486, mortality rates following rIL-1α and rTNFα were 33% (10/30) and 7% (2/30), respectively.

Total protein breakdown rate, expressed as tyrosine release, was increased by 46% and myofibrillar protein breakdown rate, expressed as 3-MH release, was increased more than two-fold following administration of rIL-1α (Fig. 1). Treatment with RU 38486 did not alter the effect of rIL-1α on total or myofibrillar protein breakdown rates (Fig. 1). The glucocorticoid receptor antagonist did not affect protein breakdown rates in control injected rats.

Following administration of rTNFα, total and myofibrillar protein breakdown rates were increased by approximately 40% and 60%, respectively (Fig. 2). This increase in protein breakdown rates was completely blocked by RU 38486 (Fig. 2).

Plasma corticosterone levels were increased 30 min after rIL-1α or rTNFα administration and remained elevated for at least 4 h (Fig. 3). The response of plasma corticosterone levels was similar to the two cytokines.

Discussion

The present results confirmed previous studies from this and other laboratories showing that both IL-1α and TNFα can induce muscle proteolysis when administered to experimental animals. The study also showed that the increase in muscle protein breakdown, induced by rIL-1α, was not affected by RU 38486, whereas TNF-induced muscle proteolysis was completely blocked by the glucocorticoid receptor antagonist.
suggest that TNF-induced muscle protein breakdown is mediated by glucocorticoids and that IL-1 stimulates muscle protein breakdown by other mechanism(s). Muscle protein synthesis was not determined in the present study because previous reports suggest that IL-1 and TNF do not regulate protein synthesis in skeletal muscle.\(^5\)\(^4\)

RU 38486 is a potent glucocorticoid receptor antagonist with no agonist activity even at high concentrations.\(^1\)\(^3\) The effectiveness of RU 38486 to block glucocorticoid receptors in different tissues, including skeletal muscle, was demonstrated previously.\(^1\)\(^7\) At a dose of 5 mg kg\(^{-1}\) body weight, RU 38486 blocked approximately 90% of glucocorticoid receptors in rat muscle 2h after administration.\(^1\)\(^7\) Chronic administration of the blocker inhibited dexamethasone-induced muscle atrophy.\(^1\)\(^7\) A dose of 15 mg kg\(^{-1}\) body weight given over 16 h, identical to the protocol used in the present study, completely prevented the proteolytic effect in rat muscle seen following injection of 200 mg kg\(^{-1}\) body weight of corticosterone.\(^4\)

The present result of glucocorticoid independent stimulation of muscle protein breakdown following administration of rIL-1\( \alpha \) is in line with a recent study from our laboratory.\(^7\) Because in other studies IL-1 did not increase muscle protein degradation when added directly to incubated muscles, even at high concentrations,\(^5\)\(^-\)\(^8\) it is not likely that muscle proteolysis following the administration of IL-1 in vivo reflects a direct effect on muscle. It is not known at present by which mechanism(s) IL-1 stimulates protein degradation in skeletal muscle. It may be speculated that catabolic hormones other than glucocorticoids, fever, haemodynamic changes and other steroid independent effects induced by IL-1 may account for its metabolic effects in muscle tissue.

In contrast to IL-1, the current study suggests that TNF requires the action of glucocorticoids to induce muscle protein breakdown. These results are in line with previous reports in which TNF-induced muscle catabolism was prevented by adrenalectomy.\(^1\)\(^2\)\(^,\)\(^1\)\(^8\) The effect of RU 38486 on muscle proteolysis following TNF has not been reported previously. It should be noted that although the present results suggest that TNF-induced muscle proteolysis is mediated by glucocorticoids, the results may also be consistent with the concept that glucocorticoids act as a co-factor to TNF. Indeed, previous studies, in which TNF was administered to rats alone or in combination with corticosterone, suggest that glucocorticoids are an important cofactor to the cytokine.\(^1\)\(^8\) Regardless of whether glucocorticoids act as a co-factor or as a second mediator released by TNF, the present study confirms that glucocorticoids are required for TNF to induce muscle protein breakdown.

Although the present results suggest that IL-1 and TNF stimulate muscle proteolysis through different mechanisms, other explanations need to be considered as well. For example, if IL-1 resulted in
Although the result of IL-1 even when rats were treated with TNF and that TNF-induced release of IL-1 was mediated by glucocorticoids. Further experiments are required to resolve this issue. The interaction between IL-1, TNF and glucocorticoids is further complicated by the fact that exogenous, and possibly endogenous, glucocorticoids may affect cytokine production.22-24

The present results are important from a clinical standpoint because both IL-1 and TNF have been implicated as regulators of muscle protein breakdown, observed in the current experiments, was the result of IL-1 even when rats were treated with TNF and that TNF-induced release of IL-1 was mediated by glucocorticoids. Further experiments are required to resolve this issue. The interaction between IL-1, TNF and glucocorticoids is further complicated by the fact that exogenous, and possibly endogenous, glucocorticoids may affect cytokine production.22-24

References

1. Baracos V, Rodemann HP, Dinarello CA, Goldberg AL. Stimulation of muscle protein degradation and prostaglandin E2 release by lipoic acid in the rat. J. Clin. Invest. 1991; 88: 107-112.

2. Flores EA, Baxtigan BR, Pomposelli JJ, Dinarello CA, Blackburn GO, and Letten NW. Infusion of tumor necrosis factor in rats with acute renal failure. J. Clin. Invest. 1992; 89: 1614-1622.

3. Zemir O, Hasselgren PO, Komniki SI, Frederick JA, Higashiguchi T, Fischer JE. Evidence that tumor necrosis factor participates in the regulation of muscle proteolysis during sepsis. Arteriosclerosis 1992; 12: 170-174.

4. Zemir O, Hasselgren PO, von Allmen D, Fischer JE. The effect of interleukin-1α and the glucocorticoid receptor blocker RU 38486 on total and myofibrillar protein breakdown in skeletal muscle. J. Surg. Res. 1991; 50: 579-583.

5. Goldberg AL, Kettler JC, Fagan K, Fagan JM, Baracos V. Activation of protein breakdown and prostaglandin E2 production in rat skeletal muscle in fever is signaled by a macrophage product distinct from interleukin-1 or other known monokines. J. Clin. Invest. 1988; 81: 1378-1383.

6. Moldawer LL, Snaiderer G, Glini J, Lando B, Halazon KG. Interleukin-1 and tumor necrosis factor do not regulate protein balance in skeletal muscle. Am. J. Physiol. 1987; 253: C766-C773.

7. Hummel RP, Warner BW, Pedersen P, et al. In vitro effect of TNF, IL-1 and other monokines on skeletal muscle amino acid uptake and protein degradation. Surg Forum 1987; 38: 13-16.

8. Hasselgren PO, James JH, Benson DW, Li S, Fischer JE. Is there a circulating protein synthesis-inducing factor during sepsis? Arch. Surg. 1990; 125: 510-514.

9. Tracey KJ, Lowry SF, Fisher TY, et al. Cachectin/murine necrosis factor in septic shock and stress hormone responses in the dog. J. Surg. Gynecol. Obstet. 1987; 164: 415-422.

10. Warren RS, Starnes HF, Alecock N, Calvano S, Brennan MD. Hormonal and metabolic responses to recombinant human tumor necrosis factor in rat. In vitro and in vivo. Am. J. Physiol. 1988; 255: E1206-E1212.

11. Gwosdow AR, Kumar MSA, Bode HH. Interleukin-1 stimulation of tyrosine-phosphate-purine-adenosine axis. Am. J. Physiol. 1990; 259: E456-E470.

12. Mealy K, van Laarhoven JB, Robinson RC, Ramos J, Wilmore DW. Are the effects of tumor necrosis factor mediated by glucocorticoids? Arch. Surg. 1990; 125: 42-48.

13. Philbert D, Rui LI. RU 38486: an original multi-factor and an antiestrogen in vivo. In: Agarwal MK, ed. Adrenal Steroid Antagonists. Berlin, New York: Walter de Gruyter & Co., 1984, 77-100.

14. Hasselgren PO, Hall-Angeris M, Angeris U, Benson D, James JH, Fischer JE. Regulation of total and myofibrillar protein breakdown in rat extensor digitorum longus and soleus muscle incubated flaccid or at resting length. Biochem. J. 1990; 267: 37-44.

15. Gwosdow-Cohen A, Chen CL, Schol EL, Radiation on (RIA) of serum corticosterone in rats. Proc. Soc. Exp. Biol. Med. 1982; 170: 29-34.

16. Goodman MN. Tumor necrosis factor induces skeletal muscle protein breakdown in rats. Am. J. Physiol. 1992; 263: E595-E605.

17. Konagaya M, Bernard PA, Max SR. Blockade of glucocorticoid receptor binding and inhibition of dexamethasone-induced muscle atrophy in the rat by RU 38466, a potent glucocorticoid antagonist. Endocrinology 1986; 119: 375-380.

18. Hall-Angeris M, Angeris U, Zemir O, Hasselgren PO, Fischer JE. Interaction between corticosterone and tumor necrosis factor stimulated protein breakdown in rat skeletal muscle, similar to sepsis. Surgery 1990; 108: 460-466.

19. Menegozzi M, Ghelzzi P. Defective tolerance to the toxic and metabolic effects of interleukin-1. J. Exp. Med. 1987; 165: 966-975.

20. Nawroth PP, Cannon JG, Wolff SM, et al. Tumour necrosis factor (cachectin) induces protein breakdown in rat skeletal muscle similar to sepsis. J. Surg. Res. 1991; 50: 510-514.