Interleukin-6 Induces Skeletal Muscle Protein Breakdown in Rats (43695)

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Abstract. **In previous studies, interleukin-1 (IL-1) or tumor necrosis factor (TNF) have been demonstrated to augment skeletal muscle protein breakdown in a manner similar to that induced by bacterial endotoxin. This response to IL-1 or TNF was elicited only after they were administered to animals for various periods, as their addition *in vitro* to incubated muscles from normal untreated rats was without effect. This suggested that IL-1 and TNF may augment muscle proteolysis in an indirect fashion. Serum levels of IL-1, TNF as well as interleukin-6 (IL-6) are all elevated during infection induced by bacterial endotoxin. Both IL-1 and TNF can induce the synthesis of IL-6 by a variety of cells. Because of this, in the present report, the ability of IL-6 to stimulate skeletal muscle protein breakdown was examined. Muscle protein breakdown was evaluated by measuring the release of both tyrosine and 3-methylhistidine by incubated muscles. Pretreatment of rats with IL-6 for 6 hr induced fever and increased the release of both tyrosine and 3-methylhistidine by the extensor digitorum longus muscle. However, IL-6 did not augment muscle proteolysis when muscles from normal untreated rats were incubated in the presence of the cytokine. The data suggest that the acute treatment of animals with IL-6 can augment muscle proteolysis. Whether this response is due to a direct effect of IL-6 on the myocyte or whether it is due to the production of other mediators remains unclear.** [P.S.E.B.M. 1994, Vol 205]

The acute metabolic response to sepsis, trauma, and neoplastic diseases includes weight loss, synthesis of acute phase proteins, enhanced nitrogen excretion, and muscle wasting (1–6). There is evidence suggesting that some of these changes may be mediated by circulating polypeptides released by stimulated macrophages or lymphocytes (1–4, 6–8). In particular, it has been suggested that interleukin-1 (IL-1) and/or tumor necrosis factor (TNF) may mediate these metabolic responses.

We reported previously that treatment of rats with TNF induced muscle protein breakdown (7). This response was similar to that of rats treated with bacterial endotoxin (7), suggesting TNF may, in part, mediate induced protein breakdown during infection. On the other hand, we could not demonstrate that treatment of rats with IL-1 induced muscle protein breakdown. The effect of TNF and IL-1 on muscle protein breakdown has not been consistent. Whereas some have reported that TNF or IL-1 does not alter rat muscle protein breakdown, others have reported that these cytokines augment muscle protein breakdown (1–4, 6–10).

In our previous studies, TNF induced muscle protein breakdown only following treatment of rats with the cytokine (7). TNF was ineffective in augmenting muscle proteolysis when muscles from normal untreated rats were incubated *in vitro* with TNF. Lack of an *in vitro* response has also been reported by others (3, 4, 9). This suggested that the effects of TNF on muscle may be mediated in an indirect manner. There is some evidence that the effect of TNF on muscle may be mediated by enhanced glucocorticoid secretion (8, 9, 11). Alternatively, some of the metabolic effects of TNF may be mediated by interleukin-6 (IL-6). IL-6 is a cytokine that plays a central role in inflammation and it is produced by a variety of cells (12–14). It has now been shown that IL-6 is identical to hepatocyte stimulatory factor, B-cell stimulatory factor-2, interferon-

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Received July 21, 1993. [P.S.E.B.M. 1994, Vol 205]
Accepted October 27, 1993.

0037-9727/94/2052-0182$10.50/0

B2, hybridoma growth factor, and human myeloid differentiation-induced protein (12–14). It has also been shown that IL-1 and TNF can enhance the expression of the IL-6 gene in different cell types (12–14). Treatment of rats with TNF can also lead to a rapid rise in serum IL-6 (15). Because of this, we tested to see if IL-6 could augment muscle protein breakdown in rats. For this purpose, young rats were either pretreated with IL-6 prior to removal of muscles for incubation or muscles from normal untreated rats were incubated *in vitro* with IL-6. Muscle proteolysis was assessed by measuring the release of both tyrosine and 3-methylhistidine (7, 16).

Materials and Methods

Animals and Diets. Male Sprague-Dawley rats obtained from Charles River Breeding Laboratories (Wilmington, MA) were used. Rats were received at a body weight of 40–50 g, were housed in animal quarters maintained at 72°F with a 12-hr light (0600–1800): 12-hr dark (1800–0600) cycle, and were given free access to Purina lab chow and water until used. The rats were used when they obtained a body weight of 70–80 g.

Materials. Chemicals were reagent grade and were obtained from Sigma Chemical (St. Louis, MO) or Fisher Scientific (San Francisco, CA). Bovine serum albumin used in incubation studies was from Miles Laboratory (Kankakee, IL). Recombinant human IL-6 was obtained from Genzyme Corporation (Cambridge, MA; specific activity 1×10^7 units/mg in induction of proliferation of B9 hybridoma cells).

Evaluation of Protein Degradation. On the day prior to an experiment (at 6:00 PM), under light ether anesthesia, rats (Group A) were injected intravenously (iv) through the dorsal tail vein with IL-6 (115 μg/kg). Control rats received an iv injection of only the drug vehicle (normal saline containing 0.1% bovine serum albumin). In some experiments, rats (Group B) received an intraperitoneal (ip) injection of IL-6 (115 μg/kg) instead of an iv injection. Following IL-6 treatment, rats were returned to their cages and given free access to water but no food. The next morning, 12 hr after treatment, rats were anesthetized with pentobarbital sodium (5 mg/100 g body wt), and the extensor digitorum longus (EDL) muscle from both legs was removed, mounted on stainless steel clips to provide slight tension, and prepared for *in vitro* incubation as described previously (16). In another study, rats (Group C) received an ip injection of IL-6 (125 μg/kg) at 9:00 AM and a second injection 3 hr later. Control animals received 0.9% saline. Following injections, rats were returned to their cages and allowed only water but no food. Muscles were harvested for *in vitro* incubation 6 hr after the first injection. In a final group of studies, muscles from normal untreated rats were incubated *in vitro* with or without IL-6 (750 ng/ml).

Protein breakdown was evaluated by measuring the release of tyrosine and 3-methylhistidine by incubated muscles as described previously (7, 16). Correction for changes in pool sizes of both tyrosine and 3-methylhistidine during the incubation are accounted for. The incubation medium contained Krebs-Henseleit bicarbonate solution (pH 7.4), 0.1% albumin, 10 m*M* glucose, and 0.1 m*M* leucine. Cycloheximide (0.5 m*M*) was added to the medium in some studies (see legend to Tables I and II). When IL-6 was added *in vitro,* the albumin concentration was increased to 1%. It is suggested that this helps prevent a loss of its activity *in vitro*. The medium was gassed with 95% O_2 – 5% CO_2. Muscles were incubated at a temperature of 37°C. EDL muscles were incubated in stoppered 20 ml glass vials that contained 3 ml incubation medium. Muscles were incubated for 1 hr following a 30-min preincubation. When IL-6 was added *in vitro,* muscles were incubated for 6 hr with a change of medium after 3 hr. At the end of each incubation, the medium (2 ml) was added to tubes that contained 80 ul of 60% perchloric acid. The tubes were vortexed, placed on ice for 10 min, and centrifuged (10,000*g*) at 4°C for 20 min. The supernatants were stored at −20°C until analyzed. The muscles were removed from the clips, blotted, weighed, and frozen at −70°C until used.

Analytical Procedures. Frozen muscle tissue was minced and homogenized in 3% (v/v) perchloric acid, centrifuged (10,000*g*) at 4°C for 20 min, and the supernatant stored at −20°C until used for analysis. Tyrosine and 3-methylhistidine in perchloric acid extracts of incubation medium and muscle tissue samples were measured as described previously (7, 16).

Statistics. Mean values for treatment groups were compared with the control group using a Student's *t*-test.

Results

Metabolic Effect of IL-6. In a previous study, treatment of rats with either bacterial endotoxin, IL-1 or TNF resulted in an increase in rectal temperature and a marked decrease in serum zinc (7). To assess the metabolic response to IL-6 in the present study, rectal temperature was measured. Temperature was not elevated when measured 12 hr following one ip or one iv injection of IL-6 (control, 37.4 ± 0.1°C [$n = 6$]); however, rectal temperature was significantly increased ($P < 0.01$) following treatment of rats with two ip injections (3 hr apart) for 6 hr (control, 37.3 ± 0.1°C vs IL-6, 38.4 ± 0.1°C [$n = 4$]).

Effect of IL-6 on Skeletal Muscle Protein Degradation. The IL-6 preparation used for these studies was metabolically effective as it induced fever. To determine whether muscle protein breakdown was likewise affected, tyrosine and 3-methylhistidine release

Table I. Effect of Interleukin-6 on Skeletal Muscle Protein Degradation[a]

Group	Route of IL-6 administration	Time muscles harvested (hr)	Tyrosine release (nmol/hr/g muscle)	3MH release
A. Control (4)	iv (once)	12	179 ± 14	2.32 ± 0.19
IL-6 (4)			200 ± 16	2.74 ± 0.22
B. Control (4)	ip (once)	12	185 ± 15	2.50 ± 0.20
IL-6 (6)			205 ± 20	2.75 ± 0.30
C. Control (4)	ip (twice)	6	180 ± 9	2.45 ± 0.19
IL-6 (6)			207 ± 10[b]	3.43 ± 0.27[b]

[a] Values are means ± SE. The number of rats in each group is in parentheses. IL-6, interleukin-6; ip, intraperitoneal; iv, intravenous; 3MH, 3-methylhistidine. Cycloheximide was present in the incubation medium. See Materials and Methods for other details.
[b] $P < 0.05$ vs control group.

by muscles removed from rats treated with IL-6 and incubated *in vitro* was assessed. As shown in Table I, muscle protein breakdown was not altered when rats were treated with one ip or one iv injection of IL-6 for 12 hr. This regimen also did not induce fever. However, a rise in temperature may have been missed. Although not shown here, when rats were treated with bacterial endotoxin (300 μg/kg, ip) for 12 hr, muscle protein breakdown was increased by 50%–100% even though rectal temperature had returned to control levels by 12 hr. A rise in temperature, therefore, does not necessarily have to correlate with an increase in muscle proteolysis, if the former is an early response. On the other hand, the inability of one ip or one iv injection of IL-6 for 12 hr to augment muscle protein breakdown may be related to dose, or it may be due to a very short half-life of IL-6 *in vivo* (17). Because of this, we next decided to treat animals with two ip injections of IL-6 spaced 3 hr apart and harvest muscles for incubation at 6 hr. This regimen resulted in induction of fever and as shown in Table I, it also led to a rise in muscle protein breakdown. Compared with control muscles, IL-6 treatment for 6 hr increased the release of tyrosine and 3-methylhistidine by 15% and 40%, respectively. The effect of IL-6 on tyrosine release was small and was significant in Group C (Table I vs Groups A and B) because of the smaller standard error. This may suggest that IL-6 augmented the breakdown specifically of myofibrillar proteins. We next determined if IL-6 could directly increase muscle proteolysis. For this purpose, muscles from normal untreated rats were incubated in the absence or presence of IL-6 (750 ng/ml) for 6 hr, with a change of fresh medium at 3 hr. However, as shown in Table II, incubation of muscles in the presence of IL-6 failed to augment muscle protein breakdown.

Table II. Effect of Interleukin-6 on Skeletal Muscle Protein Breakdown *in Vitro*

Group	Tyrosine release	3-methylhistidine release
	(nmol/hr/g muscle)	
Control (4)	94 ± 8	1.44 ± 0.12
Interleukin-6 (4)	100 ± 3	1.28 ± 0.10

Values are means ± SE. The number of rats in each group is in parentheses. Muscles from untreated normal rats were incubated for 6 hr with or without interleukin-6 (750 ng/ml) for 6 hr. Cycloheximide was omitted from the incubation medium to eliminate any toxic effect it may have during a longer incubation. See Materials and Methods for other details.

Discussion

Previous studies have demonstrated that, during sepsis or after administration of bacterial endotoxin in animals, there is a large increase in skeletal muscle protein breakdown (2, 6, 7, 11). This response may be mediated, in part, by glucocorticoids (8, 9, 11), or it may be mediated by the synthesis of various cytokines, especially IL-1 and TNF (1–4, 6–8). Treatment of animals with either IL-1 or TNF can augment muscle protein breakdown (7, 9). However, IL-1 and TNF may induce muscle protein breakdown in an indirect manner, as they did not augment muscle proteolysis when incubated with muscle *in vitro* (3, 4, 7, 10).

There is also evidence that another cytokine, IL-6, may play a role in muscle protein breakdown during infection. Serum levels of IL-1, TNF as well as IL-6 are elevated in animals treated with bacterial endotoxin (18). In addition, IL-6 in serum is elevated in patients with burn injuries and following surgery (19, 20). Both of these conditions can lead to augmented body protein breakdown (21, 22). In addition, it has been shown that IL-6 synthesis can be induced by both IL-1 or TNF (12–15). Because of this, in the present study we tested the ability of IL-6 to induce muscle protein breakdown. When an appropriate injection regimen was used, we demonstrated that IL-6 can augment muscle protein breakdown *in vivo*. However, IL-6 failed to augment muscle proteolysis when added *in vitro*. This response is similar to that of IL-1 and TNF which increased muscle proteolysis *in vivo* but not *in vitro* (3, 4, 7, 9).

IL-1, IL-6, and TNF are multifunctional cytokines produced by a variety of cells and involved in the regulation of the immune response, hematopoiesis, and inflammation (12–14). Their functions are widely overlapping and each shows its own characteristic proper-

ties. Cellular receptors for IL-1, IL-6, and TNF have been characterized (12–14), but their presence in muscle is not clear. If their presence in muscle is lacking, this could explain their inability to augment muscle proteolysis *in vitro*. On the other hand, since IL-1, IL-6, and TNF all induced muscle protein breakdown *in vivo* (7, 9), it may seem more reasonable to predict that there is a common mediator(s) produced by nonmuscle cells in response to the cytokines that triggers muscle proteolysis. Glucocorticoids may mediate some of these effects but not all (9). Alternatively, the failure of IL-6 to augment muscle proteolysis *in vitro* may indicate that factors (i.e., glucocorticoid, IL-1, TNF) in addition to IL-6 must be present to elicit a proteolytic response. This has not been tested.

In conclusion, IL-1, IL-6 and TNF have now been demonstrated to augment muscle proteolysis *in vivo*. The data forthcoming from the present study as well as others suggest that these cytokines may augment muscle protein breakdown in an indirect manner which may ultimately be caused by the synthesis of a common mediator produced in nonmuscle cells. This remains unclear.

This work was supported in part by a research grant from the American Cancer Society (CN-61).

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