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**Research Article**

Systemic infection with Streptococcus pneumoniae produced atrophy, decreased twitch and tetanic tension, and altered intracellular electrolyte composition in rat skeletal muscle. Cathepsin B activity was selectively elevated early in the course of illness. Luepeptin, a cathepsin B inhibitor, and indomethacin, a prostaglandin synthesis inhibitor, prevented muscle atrophy and impaired contractility. Indomethacin, but not leupeptin, prevented the intracellular electrolyte changes. Acetaminophen reduced fever but did not prevent muscle atrophy, impaired contractility, or altered intracellular electrolytes. Muscle wasting and impaired contractility associated with sepsis may involve selective prostaglandin stimulation of cathepsin B activity. Intracellular electrolyte changes may involve prostaglandin synthesis but do not require cathepsin B activation.



# Rapid Publication

## Inhibitors of Prostaglandin Synthesis or Cathepsin B Prevent Muscle Wasting Due to Sepsis in the Rat

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**Abstract.** Systemic infection with *Streptococcus pneumoniae* produced atrophy, decreased twitch and tetanic tension, and altered intracellular electrolyte composition in rat skeletal muscle. Cathepsin B activity was selectively elevated early in the course of illness. Leupeptin, a cathepsin B inhibitor, and indomethacin, a prostaglandin synthesis inhibitor, prevented muscle atrophy and impaired contractility. Indomethacin, but not leupeptin, prevented the intracellular electrolyte changes. Acetaminophen reduced fever but did not prevent muscle atrophy, impaired contractility, or altered intracellular electrolytes. Muscle wasting and impaired contractility associated with sepsis may involve selective prostaglandin stimulation of cathepsin B activity. Intracellular electrolyte changes may involve prostaglandin synthesis but do not require cathepsin B activation.

### Introduction

Systemic bacterial or viral infection produces potassium depletion and clinical muscle wasting (1). Similarly, the common symptom of muscle aching and weakness with febrile illness may be associated with accelerated proteolysis in skeletal muscle (2). Several recent studies have suggested a mechanism by which systemic infection induces muscle protein degradation. Leukocytic pyrogen is a polypeptide released by phagocytic white blood cells (3) which, when incubated with muscle, stimulated synthesis of a specific prostaglandin (PG),[1] prostaglandin $E_2$ (4), and stimulated muscle protein degradation (2). Clowes et al. (5) isolated a polypeptide from the serum of septicemic patients that stimulated skeletal muscle proteolysis in vitro. This polypeptide was thought to be an active metabolite of leukocytic pyrogen (2, 5). We studied the effect of systemic bacterial infection on skeletal muscle protease activity, and the ability of indomethacin, a potent PG synthesis inhibitor (6), or leupeptin, an inhibitor of lysosomal thiol proteases and calcium-activated protease (7), to reduce muscle wasting associated with sepsis.

### Methods

Male Sprague-Dawley rats were individually housed and their food and water consumption were measured daily. The animals received a 12:12 light/dark photoperiod. Rats were arbitrarily divided into groups of 14. Each rat received a single subcutaneous injection of $2 \times 10^5$ virulent (septic, S) or heat-killed (control, C) *Streptococcus pneumoniae*. In preliminary experiments, 48 rats received this inoculum of live bacteria with a mortality rate of 10% at 3 d, 46% at 5 d, and 96% at 8 d. Some groups of rats injected with live bacteria received intraperitoneal injections four times daily of leupeptin, 2.5 mg/kg initial body mass (S-L), or of indomethacin, 5 mg/kg initial body mass (S-I); or oral doses four times daily of acetaminophen, 7.5 mg/kg initial body mass (S-A). These treatments were started at the time of inoculation with live bacteria. Preliminary experiments in control rats indicated that these doses of leupeptin, indomethacin, and acetaminophen did not alter the normal pattern of weight gain, muscle contractile properties, or muscle or plasma electrolytes. C, S-L, S-I, and S-A rats were fed the same amount of rat chow consumed by S rats on each day after inoculation (pair-fed). Animals were killed 3 d after inoculation. One S rat, 2 S-L rats, 2 S-I rats, and 1 S-A rat died before 3 d. The techniques for measuring intracellular sodium and potassium, and twitch and tetanic tension were previously described (8).

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1. *Abbreviations used in this paper:* EDL, extensor digitorum longus; C, control; PG, prostaglandin; S, septic; S-L, S-leupeptin; S-A, S-acetaminophen; S-I, S-indomenthacin; S-L, septic-leupeptin.

Cathepsin B activity was determined by spectrophotometric measurement of naphthylamine, which is produced from the hydrolysis of benzoyl-D,L-arginine-2-naphthylamide by cathepsin B; the technique was described by Barrett and Heath (9). Cathepsin D activity was assayed by using bovine hemoglobin as the substrate, according to the method of Barrett and Heath (9). Calcium-activated protease activity was measured according to a modification of the technique of Kar and Pearson (10), and was calculated as the difference in the rate of degradation of casein-yellow between samples incubated at 37°C for 1 h with 1 mM $CaCl_2$ and samples containing 5 mM EGTA. Proteolysis was assayed spectrophotometrically as the supernatant's change in absorption at 428 nm. A unit of activity was defined as that amount of enzyme that caused an absorbance change of 0.001. Protein content was determined by the Folin-Lowry technique (11). The values of $P$ for significance of the difference between groups was determined by the two-tailed Dunnets $t$ test (12). Values are given as mean±SE.

## Results

Control animals showed a normal diurnal temperature pattern, whereas rats infected with virulent bacteria developed fever within 12 h of inoculation (Fig. 1). At necropsy, the S rats had multiple foci of *S. pneumoniae* infection in the lungs. However, there was no histologic evidence of inflammation, infection, or regenerating fibers in the fast-twitch extensor digitorum longus (EDL) or slow-twitch soleus muscles. C rats had no histologic signs of infection and blood and urine cultures were negative. Sepsis resulted in significant loss of body weight and muscle mass (Fig. 2). However, since the water content of muscle was not altered by infection (C, 814±20 ml/kg; S, 788±19 ml/kg muscle mass), the reduction in muscle mass was not due to dehydration. The daily food intakes were as follows. Day 1: C, 14.1±0.6 g; S, 14.4±0.7 g; S-L, 13.4±0.6 g; S-I, 12.8±0.5 g; S-A, 13.6±0.6 g. Day 2: C, 11.7±0.4 g; S, 12.1±0.5 g; S-L, 11.1±0.3 g; S-I, 11.3±0.5 g; S-A, 11.1±0.4 g. Day 3: C, 9.9±0.3 g; S, 10.1±0.4 g; S-L, 10±0.3 g; S-I, 10±0.3 g; S-A, 9.9±0.4 g. Intracellular potassium was reduced and sodium was increased by infection. In addition, twitch and tetanic tension were decreased by sepsis (Fig. 3).

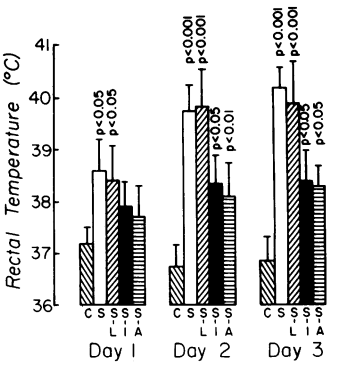


*Figure 1.* The effect of sepsis on the maximal rectal temperature recorded during the light photoperiod on each day after inoculation with $2 \times 10^5$ virulent (S) or heat-killed (C) *S. pneumoniae*. Some rats injected with live bacteria received leupeptin (S-L), indomethacin (S-I), or acetaminophen (S-A). On days 2 and 3 the body temperature of S-I and S-A rats was lower than that of S rats ($P < 0.005$). The $P$ values in the figure refer to differences from control. For all figures: $n = 14$ for C, $n = 13$ for S, $n = 12$ for S-L, $n = 12$ for S-I, and $n = 13$ for S-A.

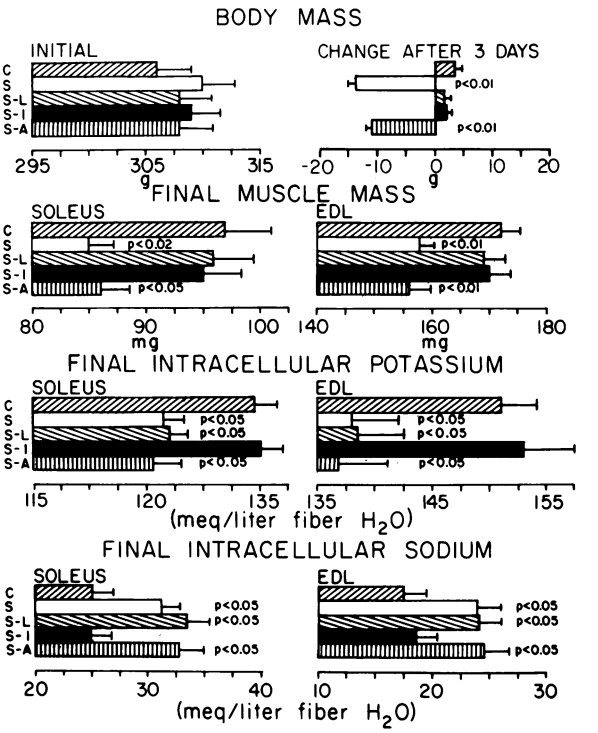


*Figure 2.* The effects of sepsis on body and muscle weight and intracellular electrolyte composition. Final values refer to those obtained 3 d after inoculation.

Degradation of myofibrillar protein by proteases such as cathepsin B and D, or calcium-activated protease could produce muscle atrophy and impaired force generation capacity (9, 13). Infection increased cathepsin B activity by 300% in EDL and 210% in soleus, however the activities of cathepsin D and calcium-activated protease were not changed by sepsis (Fig. 4).

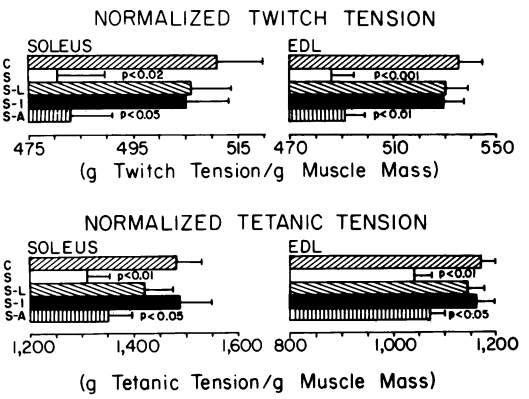


*Figure 3.* The effect of sepsis on force generating capacity. Twitch and tetanic tension were measured in vitro at 37°C 3 d after inoculation.

Cathepsin B activity rose early in the course of infection. 8 h after inoculation with virulent bacteria, cathepsin B activity was increased 180% in soleus and 260% in EDL. In rat skeletal muscle leupeptin can inhibit cathepsin B activity without stimulating protein synthesis (7). Administration of leupeptin prevented activation of cathepsin B (Fig. 4), muscle atrophy (Fig. 2), and reduction in normalized twitch and tetanic tensions (Fig. 3) associated with infection. However, leupeptin did not prevent fever (Fig. 1) or alterations in muscle electrolyte composition (Fig. 2). At necropsy, 72 h after inoculation, leupeptin-treated animals had multiple foci of *S. pneumoniae* infection in the lungs.

If PG stimulates protease activity, then inhibition of PG synthesis should prevent muscle atrophy and reduction in twitch and tetanic tension. Indomethacin treatment prevented the changes in muscle mass (Fig. 1), force generating capacity (Fig. 3), intracellular electrolyte composition (Fig. 2), and cathepsin B activity (Fig. 4) associated with sepsis. Indomethacin did not prevent systemic infection. At necropsy, indomethacin-treated animals had multiple foci of infection in the lungs. However, indomethacin did reduce fever in infected animals (Fig. 1). To determine if any of the effects of indomethacin were due to its antipyretic action, infected rats were treated with acetaminophen, a potent antipyretic drug that has little effect on PG synthesis outside the central nervous system (14). Acetaminophen reduced fever to the same extent as indomethacin (Fig. 1). However, acetaminophen treatment did not prevent the muscle changes associated with infection (Figs. 1–3). Thus, the effects of indomethacin were probably due to inhibition of PG synthesis and the subsequent activation of cathepsin B, rather than due to its antipyretic action.

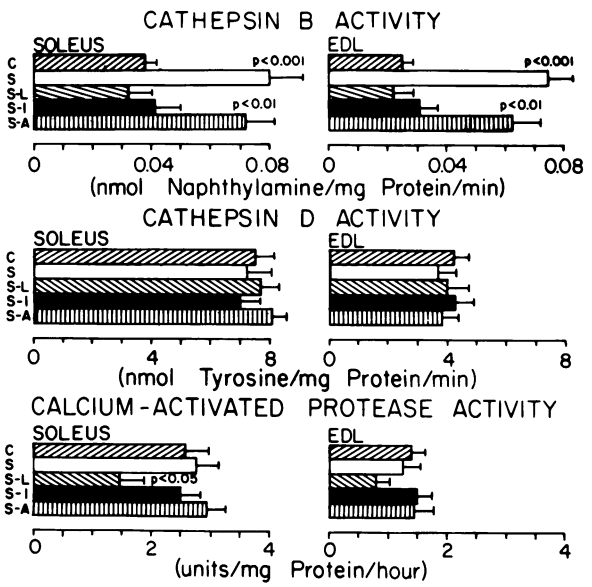


*Figure 4.* Effect of sepsis on protease activity measured 3 d after inoculation.

## Discussion

The data suggest that muscle wasting and impaired contractility associated with infection are mediated by PG via pathways that involve activation of intracellular proteases including cathepsin B, while alteration in the intracellular electrolyte composition may not require protease activation. The EDL and soleus were not invaded by inflammatory cells, so the elevated protease activity probably represented activation of muscle intracellular proteases. In this study, cathepsin D and calcium-activated protease were not activated by infection, suggesting that PG did not simply produce a generalized increase in muscle protease activity. These data support the suggestion by Rodemann et al. (15) that cathepsin B, and not calcium-activated protease, is important in regulating proteolysis in skeletal muscle.

Indomethacin and leupeptin were able to limit the muscle atrophy and impairment of contractile force associated with infection, possibly because leupeptin inhibited lysosomal thiol proteases, including cathepsin B, and indomethacin prevented the PG formation. In cell-free systems, leupeptin inhibits the lysosomal thiol cathepsins B, H, and L, and the cytoplasmic calcium-activated protease (7, 9, 13). Leupeptin is not a potent inhibitor of calcium-activated protease in intact rat muscle (4). The activity of calcium-activated protease in soleus but not EDL was reduced by leupeptin (Fig. 4). Consequently, the ability of leupeptin to prevent wasting of the EDL could not be attributed to reduced calcium-activated protease activity.

Indomethacin prevented muscle breakdown while acetaminophen, a frequently used antipyretic agent, was ineffective. Therefore, as suggested by Baracos et al. (2), PG synthesis inhibitors, but not acetaminophen, may be clinically useful in limiting the muscle wasting associated with infection.

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