Brief Definitive Report

CACHECTIN/TUMOR NECROSIS FACTOR MEDIATES CHANGES OF SKELETAL MUSCLE PLASMA MEMBRANE POTENTIAL

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Invasive bacterial infection in mammals often stimulates a fatal syndrome of hypotension, organ dysfunction and somatic tissue edema (1). This systemic response to critical illness in man is associated with somatic cell injury, as measured by a decrease in skeletal muscle transmembrane potential difference (E_m), increased cellular sodium and water levels, and depletion of cellular potassium stores (2). These alterations in plasma membrane function are not temporally related to inadequate tissue perfusion or to depletion of high-energy phosphate stores (3). To date. no direct mediator for this deterioration of plasma membrane and electrolyte homeostasis has been identified.

Recent work (4, 5) has shown that cachectin/tumor necrosis factor (TNF) is an important mediator of the lethal effects of endotoxin/LPS (6), and is produced in large quantities by macrophages exposed to endotoxin (7, 8). High-affinity receptors for cachectin (7) stimulate altered cellular energy metabolism both in vivo and in vitro, but the evaluation of target tissues thus far has not demonstrated any adverse influence on normal cell viability. Several host tissues possess cachectin receptors (muscle, adipose, liver) (7) and exhibit extracellular responses at low receptor occupancy, a characteristic of many systemic mediators.

Since passive immunization against cachectin confers a survival advantage in endotoxin/LPS-treated animals (6), we hypothesized that this monokine might affect skeletal muscle plasma membrane function during endotoxemia and shock. In this study, we observed that cachectin stimulated skeletal muscle fiber depolarization in an isolated muscle model. The plasma membrane response was prevented by pretreatment of cachectin with specific antibody to cachectin, and was not due to endotoxin/LPS. Measurement of muscle E_m in vivo showed a similar decline after cachectin infusion.

Materials and Methods

Recombinant human cachectin was prepared from a yeast expression system by previously described methods (4) and diluted in Krebs-Ringer-bicarbonate buffer (KRB) to the

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final concentrations indicated for each experiment. A neutralizing mAb (SW18.1) to human cachectin was prepared in mice and diluted to an initial concentration of 0.5 mg/ml. Cachectin (10 nM/liter) was incubated with mAb (diluted 1:100) in 3 ml KRB for 1 h at 0°C and 1 h at 37°C. LPS (*E. coli* strain 0127:B8; Difco Laboratories, Detroit, MI) was diluted to 0.7 ng/ml in KRB.

In all experiments, we used female Sprague-Dawley rats weighing 175–250 g, and fed ad libitum. During pentobarbital anesthesia, the extensor digitorum longus and soleus muscles were excised with the tendons intact. The specimens were quickly transferred to 20-ml vials containing 3 ml KRB, pH 7.40, with insulin 0.1 U/ml, glucose (5 mM), and chloramphenicol (0.3 μg/ml). The electrolyte content of the medium was: NaCl, 119 mM; KCl, 5 mM; $CaCl_2$, 3 mM; KH_2PO_4, 1 mM; $MgSO_4$, 2 mM; $NaHCO_3$, 25 mM. Muscles were gassed continuously with 95% O_2/5% CO_2 except during measurement of E_m. After an initial period, which allowed time for cellular recovery and equilibration (30 min), muscles were transferred to fresh, oxygenated medium, and the basal muscle fiber E_m was recorded at room temperature. Specimens were then transferred to freshly oxygenated medium containing additives required for each experiment, and E_m was again determined.

Membrane potentials were measured with modified Ling-Gerard microelectrodes prepared on a vertical pipette puller from borosilicate glass capillary tubes to a tip diam of ~0.1 μm. Electrodes were filled with a solution of 3 M KCl, and the tip resistance was measured. Only those tips with resistance of 5–15 MΩ were used. During fiber impalements, tip resistance changed by <10%.

The measurement of E_m was standardized by the following technique. The microelectode tip was positioned vertically above the muscle, midway between the tendons, with a Leitz micromanipulator. The electrode was advanced until a superficial fiber was penetrated, as judged by a sharp deflection on the chart recorder. The difference between the Ag/AgCl reference electrode in the medium and the steady-state recording of potential for 10 s, free of high-frequency interference, was considered to be the resting membrane potential of the fiber. The electrode was then advanced until a second fiber was penetrated, after which the tip was withdrawn and moved laterally. This process was repeated until membrane potentials were measured in ~35 individual fibers. No measurement was rejected on the basis of its absolute magnitude.

E_m measurement in vivo was performed on the exposed hindlimb adductor muscles by previously described methods (3).

Statistical analysis was by Student's paired *t* test as individual specimens were compared to the measured, basal recording of E_m before the experimental measurement with the additives indicated.

Results and Discussion

The mean E_m for extensor digitorum longus (−70.4 ± 1.4 mV) and soleus (−71.1 ± 1.0 mV) muscles did not differ significantly, and therefore the results obtained in those specimens determined under similar conditions were analyzed together. The muscle E_m during basal incubation conditions (−71.6 ± 1.0 mV) agrees closely with previous reports using a similar model (9).

Exposure to cachectin (10 nM/liter) was associated with a decline of muscle fiber E_m (−58.6 ± 1.0 mV) that was highly significant ($p < 0.001$) compared to the muscle E_m of each specimen during basal incubation conditions (Fig. 1). Experience with this model has demonstrated cell viability for 2 h, as shown by stable intracellular ATP stores, ability to contract, and maintenance of E_m (10). In agreement with these findings, the basal recordings of E_m varied little throughout the experimental period (Fig. 1). Cachectin induced significant depolarization of muscle fiber E_m independent of the incubation time. The magnitude of depolarization initiated by cachectin did not change significantly between incubation intervals. Although we did not determine the precise time required for

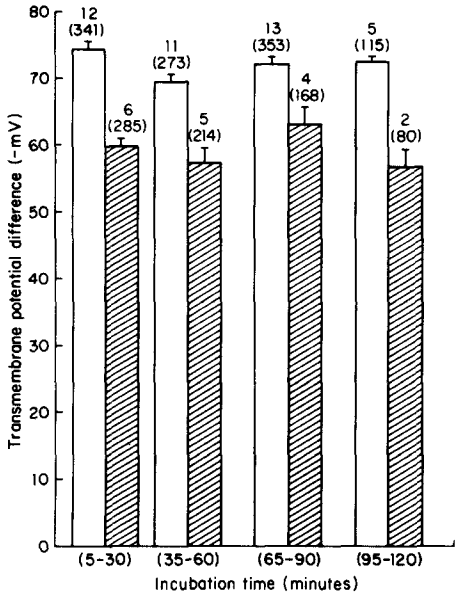

FIGURE 1. Effect of incubation conditions on transmembrane potential and response to cachectin. The membrane potentials in muscle fibers were recorded during basal incubation conditions (open bars) and after exposure to cachectin (striped bars). Data are the mean ± SE (mV), calculated on the basis of the number of muscles (n), not on the total number of impalements (shown in parentheses above bars). The alteration of E_m after exposure to cachectin was significant at all time intervals tested ($p < 0.001$).

cachectin to induce depolarization of individual cells, cellular impalements began within 5 min after exposure to the monokine, and were completed within 20 min. Thus, the results reflect the acute response of the muscle fiber to cachectin.

The depolarization of muscle E_m by cachectin was inhibited by preincubation of the monokine with a neutralizing mAb (Fig. 2). The ability of cachectin to affect muscle fiber E_m was also lost after heat-denaturation of the monokine. Under the conditions of our assay, LPS by itself at supraphysiologic concentrations (0.7 ng/ml) also failed to mediate any alteration of muscle fiber E_m.

The addition of cachectin to suspensions of skeletal muscle induced plasma membrane polarization in a dose-related manner (Fig. 3). A saturating concentration of cachectin (e.g., that concentration required to produce maximal effect) was acheived at 0.1 nM/liter. The amount of cachectin that affected E_m of muscle in vitro (>0.01 nM/liter) is comparable to the physiologic levels produced in vivo after exposure to endotoxin (8). Maximal plasma cachectin activity is achieved 2 h after endotoxin infusion (8). This response time agrees closely with the temporal delay of decreased muscle fiber E_m in dogs given gram-negative bacteremia (G. T. Shires, unpublished observation).

After i.v. injection of cachectin in rats, no evidence of tetany or muscle dysfunction was apparent (11). For 1 h after injection of a lethal dose of cachectin, animals were ambulatory and breathing normally. However, as Table I indicates, cachectin mediated a decline in skeletal muscle E_m during this time period. The decrease of cellular E_m in vivo may represent the primary signal that initiates the abnormalities of cellular metabolism following cachectin infusion.

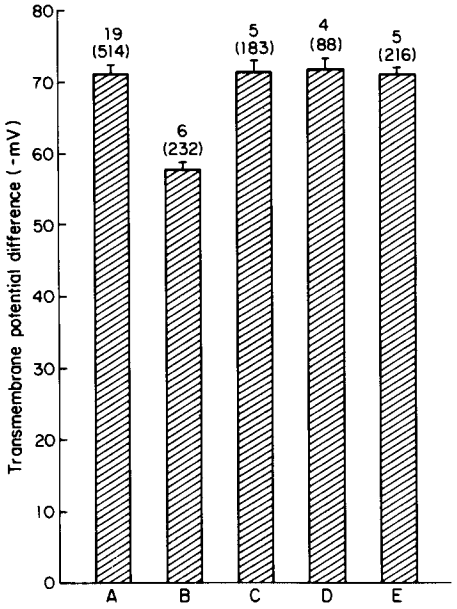

FIGURE 2. Specificity of cachectin-induced depolarization of muscle membranes. Muscles were incubated and then transferred into fresh media containing one of the additives indicated. (*A*) Basal incubation conditions, (*B*) cachectin (10 nM/liter) ($p < 0.001$ compared to basal levels), (*C*) cachectin pretreated with mAb. Antibody alone diluted 1:100 in KRB did not exhibit any independent effect on muscle E_m (data not shown). (*D*) Heat-denatured cachectin (10 nM/liter) (*E*) LPS (0.7 ng/ml). Data are mean ± SE (mV), presented as in Fig. 1.

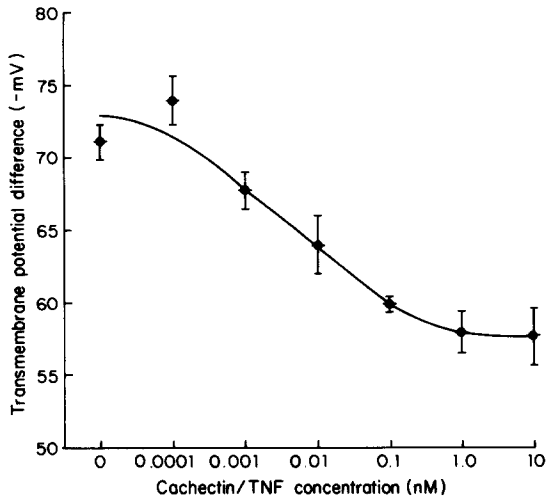

FIGURE 3. Estimation of dose/response effect of cachectin on muscle E_m. Basal membrane potentials were measured for 30 muscle specimens, which were then randomly assigned into one of six groups for exposure to cachectin at the concentrations (nM) shown. Vertical bars indicate the SE, calculated as in Fig. 1. The differences as compared to paired controls were significant at concentrations of 0.01 nM ($p < 0.05$), 0.1 nM ($p < 0.05$), 1.0 nM ($p < 0.001$), and 10 nM ($p < 0.001$).

TABLE I
Effect of Cachectin on Muscle Fiber E_m in Vivo

Cachectin dose	E_m	
	Before	After
mg/kg	*−mV*	
0.6	95.0 ± 1.3 (15)*	85.5 ± 1.0 (21)
1.8	93.4 ± 0.3 (70)	75.6 ± 1.6 (94)

Cachectin was infused into the vena cava over 20 min. E_m was measured before, and 1 h after infusion. Data are means ±SE.
* Number of fibers measured.

The effect of cachectin on skeletal muscle plasma membrane function is particularly interesting because observations of critically ill humans have revealed a depolarization of similar magnitude (2). While the mechanism of this depolarization remains obscure, it may be occurring in other cell types as well, to mediate the effects of cachectin on cellular metabolism (12, 13). Recent observations[1] have shown that cachectin is capable of increasing the rate of glucose uptake and stimulating lactate production by muscle cells in culture. The present study suggests that this phenomenon may occur in response to the direct effect of cachectin on the maintenance of resting E_m.

The cachectin-induced change in membrane potential of muscle offers an explanation for the increased sodium space and fluid retention that occurs in shock, since 40% of lean body mass would be affected. Moreover, systemic alterations of host metabolism (metabolic acidosis, hyperkalemia, and elevated blood lactate levels) may be evoked by the cellular response to this monokine. The ability of the mAb against cachectin to prevent the reduction of muscle E_m points to its potential utility in the treatment of septic shock.

Summary

Lethal infections are associated with cellular dysfunction as evidenced by a decrease in the resting transmembrane potential difference (E_m) of skeletal muscle fibers. Endotoxin stimulation of macrophages evokes production of cachectin, a protein that has been implicated as a mediator of the lethal effects of endotoxemia. In the present study, rat skeletal muscle fiber E_m decreased when incubated with recombinant human cachectin. The reduction of E_m induced by cachectin occurred in a dose-related fashion and was inhibited by mAb against the monokine. Infusion of cachectin induced a decline of skeletal muscle E_m in vivo, and suggests that cachectin may acutely mediate alterations of skeletal muscle membrane function after infection.

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[1] Lee, M. D., A. Zentella, W. Vine, P. H. Pekala, and A. Cerami. Effect of endotoxin induced monokines on glucose metabolism in the muscle cell line L6. Manuscript in preparation.

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