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...
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; CaCl₂, 3 mM; KH₂PO₄, 1 mM; MgSO₄, 2 mM; NaHCO₃, 25 mM. Muscles were gassed continuously with 95% O₂/5% CO₂ 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 microelectrode 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 (\(-70.4 \pm 1.4\) mV) and soleus (\(-71.1 \pm 1.0\) 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 (\(-71.6 \pm 1.0\) 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 \( E_m \) (\(-58.6 \pm 1.0\) 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
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 achieved 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

| Cachectin dose (mg/kg) | Before | After |
|-----------------------|--------|-------|
| 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 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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