LIPOPOLYSACCHARIDE AND SERUM SYNERGISTICALLY
STIMULATE ORNITHINE DECARBOXYLASE IN
CHINESE HAMSTER OVARY CELLS

ANNE RISSA L. GREENFIELD, STEVEN M. TAFFET, AND MARI K. HADDOX

Departments of Pharmacology and Internal Medicine, University of Texas
Medical School at Houston, Houston, Texas 77025

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SUMMARY

Lipopolysaccharide (LPS), the active component of bacterial endotoxin, caused no
significant increase in ornithine decarboxylase (ODC) activity in serum-starved, Chinese
hamster ovary fibroblasts. However, concurrent addition of LPS with 10% fetal bovine
serum caused a synergistic 30 to 40-fold increase in enzyme activity as compared to
the 10 to 20-fold increase seen after addition of serum alone. This synergism was not
due to an alteration in the time course of enzyme induction after serum addition. The
LPS-induced synergy of ODC induction by serum was inhibited by the concurrent
addition of the specific LPS-antagonist, Polymyxin B.

Key words: lipopolysaccharide; ornithine decarboxylase; Chinese hamster ovary.

INTRODUCTION

Lipopolysaccharide (LPS), the active component of gram negative bacterial endotoxin, has been shown in vitro to activate macrophages (1) and to stimulate chick and murine embryonic fibroblasts mitogenically (2,3) as well as murine B lymphocytes (4). The site within the growth process at which this action is imposed has not been identified. In the past, LPS stimulation of fibroblast growth has been examined in density-arrested cells maintained in conditioned medium containing serum (2,3); these studies did not control for endogenous endotoxin in media and sera or the possibility of interaction between LPS and serum factors. Ornithine decarboxylase (ODC), a rate-limiting enzyme in polyamine biosynthesis, increases in all growth responses studied to date, including hormone and drug-induced tissue growth and cell cycle traverse in cell culture (5,6), and is essential to the growth process (7,8). Lipopolysaccharide has been shown to increase ODC activity in macrophages, its presumed target cell in vivo (9,10). We proposed to determine if the comitogenic action of LPS on fibroblasts included an effect exerted early in the growth process at the level of ODC induction. To examine this proposal we determined whether ODC in Chinese hamster ovary (CHO) cells could be stimulated by LPS in the absence and presence of serum when cells were grown under endotoxin-free conditions.

MATERIALS AND METHODS

McCoy's 5A was prepared from powdered mix (GIBCO, Grand Island, NY) using pharmacological grade pyrogen-free water. Fetal bovine serum (FBS) was obtained from Sterile Systems, Inc. (Logan, UT) and GIBCO. All tissue culture reagents (except where shown to be positive) were tested as negative for endotoxin by the Limulus amoebocyte lysate assay (Associates of Cape Cod, Woods Hole, MA) to a sensitivity of 0.2 ng/ml. The FBS purchased from GIBCO contained >10 ng/ml of endotoxin whereas Sterile System FBS was tested as negative. Bacterial LPS from E. coli:O11:B4 was purchased from Sigma Chemical Company (St. Louis, MO). The LPS was dissolved in McCoy's medium at 1.0 mg/ml. Polymyxin B sulfate (PMB) (Sigma) was dissolved in McCoy's medium at 25 mg/ml.

Chinese hamster ovary cells (obtained from Dr. Max Costa, Houston, TX) were maintained in McCoy's 5A medium containing 10% FBS and incubated at 37°C in a humidified 95%
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air: 5% CO₂ atmosphere. Cells were subcultured by trypsinization in phosphate buffered saline containing 0.25% trypsin (Flow Laboratories, McLean, VA). For the experiments, Falcon plastic petri dishes (60 mm) (Falcon Plastics, Los Angeles, CA) were inoculated with 2 × 10⁶ cells. The cultures were grown for 24 h in complete medium, rinsed three times with serum-free medium, and incubated for an additional 48 h in serum-free medium. Plates contained approximately 1 × 10⁶ quiescent cells.

The ODC activity was measured in cell supernatant fluids as described in (11) with minor modifications. Cells were washed two times in phosphate buffered saline and harvested by scraping in 0.5 ml of 50 mM Na₂KPO₄, pH 7.2, containing 0.1 mM EDTA, 2.0 mM dithiothreitol, 5.0 mM NaF, 30 μM pyridoxal phosphate, 0.1% Brij 35, and 1.0 mM phenylmethyl-sulfonylfluoride. Two 100 μl aliquots of a 10 000 ×g supernatant solution (5 min) of each sample were incubated for 60 min at 37° C in the presence of 0.5 mM L-ornithine containing 0.5 μCi L-[1-¹⁴C]ornithine (New England Nuclear, Boston, MA). The assay was conducted in 15-ml tapered tubes fitted with Kontes rubber stoppers and center wells containing folded filter paper pretreated with 20 μl of 2 N NaOH. The reaction was terminated by the addition of 1 ml of 1 M citric acid. The incubation was continued at room temperature for another 15 min, and the CO₂ evolved was trapped as Na¹⁴CO₃ on the filter papers. The filter paper was then placed in toluene-Omnifluor and radioactivity was determined in a liquid scintillation spectrometer.

Protein determinations were performed by the method of Bradford (12), using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Medium from quiescent cells was removed and fresh medium containing the various test reagents (FBS, LPS, PMB) was added for 4 h at which time the cellular ODC activity was assessed. We have established previously that consistently measurable ODC activity was induced in CHO cells 4 h after the addition of serum.

Treatment of serum-deprived CHO cells with LPS for 4 h in the absence of any serum caused no significant increase in ODC activity (Table 1). In contrast, ODC activity increased 10 to 20-fold with the addition of 10% FBS for the same time period. The addition of LPS (1 μg/ml) in combination with 10% FBS led to a greater than cumulative effect on ODC activity (Table 1). The LPS-induced synergism resulted in a 30 to 40-fold increase in ODC activity. To determine if the apparent synergistic effect of LPS was the result of an alteration in the temporal nature of the enzyme induction, the time dependence of the enzyme increase was established. The time course of ODC activity was identical in cells treated with serum or serum and LPS (Fig. 1). No ODC activity was observed in the first 2 h after addition of serum or serum and LPS. The ODC activity was maximal at 4 h in both cases. Lipopolysaccharide synergism was observable only

![FIG. 1. Time course of ODC induction by 10% fetal bovine serum (Sterile Systems) in the presence (■) and absence (■) of LPS (1 μg/ml); effect on ODC levels of LPS alone at 1 μg/ml (■) and 10 μg/ml (■). Each value represents the average of duplicate analyses of duplicate plates.]

### TABLE 1

<table>
<thead>
<tr>
<th>The Effect of LPS on ODC Induction in CHO Cells⁴</th>
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<tbody>
<tr>
<td>ODC Activity, picomoles per hour per milligram protein</td>
</tr>
<tr>
<td>Media</td>
</tr>
<tr>
<td>61</td>
</tr>
<tr>
<td>LPS</td>
</tr>
<tr>
<td>SS</td>
</tr>
<tr>
<td>SS + LPS</td>
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<tr>
<td>GIBCO</td>
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<tr>
<td>GIBCO + LPS</td>
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</table>

⁴ ODC activity was measured 4 h after additions. Each value represents the average of duplicate analysis of duplicate plates. SS = 10% Sterile Systems fetal bovine serum. GIBCO = 10% GIBCO fetal bovine serum.