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# Effect of Lipopolysaccharide on Tumor Necrosis Factor and Prolactin Release from Rat Anterior Pituitary Cells

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**TNF-α plays a critical role in the cascade of neuroendocrine events during inflammation and septic shock. It also affects the release of pituitary hormones and acts as a growth factor in immune and nonimmune cells. The aim of the present study was to investigate the release of TNF-α from rat anterior pituitary cells and the effect of the steroid medium on its release. Cultured anterior pituitary cells from lactating rats spontaneously released TNF-α. The presence of lipopolysaccharide (LPS, 0.1 μg/mL) in the culture medium significantly increased TNF-α release and inhibited prolactin release. Chronic estrogenization of ovariectomized rats or the presence of 17 β-estradiol in the culture medium also increased TNF-α release. LPS significantly stimulated TNF-α release in all groups and abrogated the estrogen-induced prolactin release. We also investigated the effect of TNF-α on prolactin release. The presence of TNF-α (50 ng/mL) in the culture medium inhibited prolactin release from anterior pituitary cells. These data show that anterior pituitary cells in culture release TNF-α and that this release is stimulated by estrogens. Our results also indicate that LPS inhibits prolactin release in an estrogenic environment, suggesting that TNF-α could affect pituitary hormone release during endotoxemia.**

**Key Words:** TNF-α; estrogens; lipopolysaccharide; prolactin; anterior pituitary.

## Introduction

Tumor necrosis factor-alpha (TNF-α) is one of the central mediators in the pathophysiologic events following endotoxemia and sepsis *(1)*. This multifunctional cytokine is produced by monocytes, macrophages, and microglia in response to bacterial endotoxin (lipopolysaccharide [LPS]) *(2,3)*. It is also synthesized by nonimmune cells, such as uterine, ovarian, glial, and neuronal cells *(4–7)*. TNF-α exerts a great variety of effects on immune and nonimmune cells and is thought to be of physiological importance in tissue growth *(8–10)*. TNF-α is also known to mediate interactions between the immune and neuroendocrine systems *(11)*. Several cytokines and growth factors affect pituitary hormone release, and some of them are expressed in the pituitary gland itself *(12–14)*. The presence of binding sites for TNF-α and the expression of its mRNA in the anterior pituitary *(15,16)* suggest that this cytokine may play a role in the control of anterior pituitary function, thus affecting the release of pituitary hormones *(17–20)*.

The administration of LPS markedly affects pituitary secretion, and its effects are probably mediated by peripherally produced cytokines from immune cells *(21)* or by the synthesis and release of brain cytokines *(22)*. LPS stimulates corticotropin-releasing hormone (CRH) release *(23)* and suppresses luteinizing hormone-releasing hormone (LHRH) pulse generator activity *(24)*. TNF-α and interleukin-6 (IL-6) mediate the LPS-induced adrenocorticotropin hormone (ACTH) release, and both IL-1α and IL-1β are involved in the LH suppression caused by LPS *(25,26)*. In addition, it has been suggested that LPS can modulate pituitary function either by a direct effect on secretory cells *(27)* or by inducing pituitary cytokine production *(13,14)*. However, the direct effects of LPS on endocrine tissues are still poorly understood.

The present study investigated the effect of LPS on TNF-α and prolactin release from primary cultures of anterior pituitary cells. Since it has been suggested that steroids modulate the synthesis and/or activity of some cytokines *(28–30)*, we investigated the effect of estrogens and lactation on the pituitary response to LPS. We also studied the effect of TNF-α on prolactin release.

## Results

### *Effect of Lactation on TNF-α and Prolactin Release*

Anterior pituitary cells from lactating rats spontaneously released TNF-α to the culture media. When the cells were

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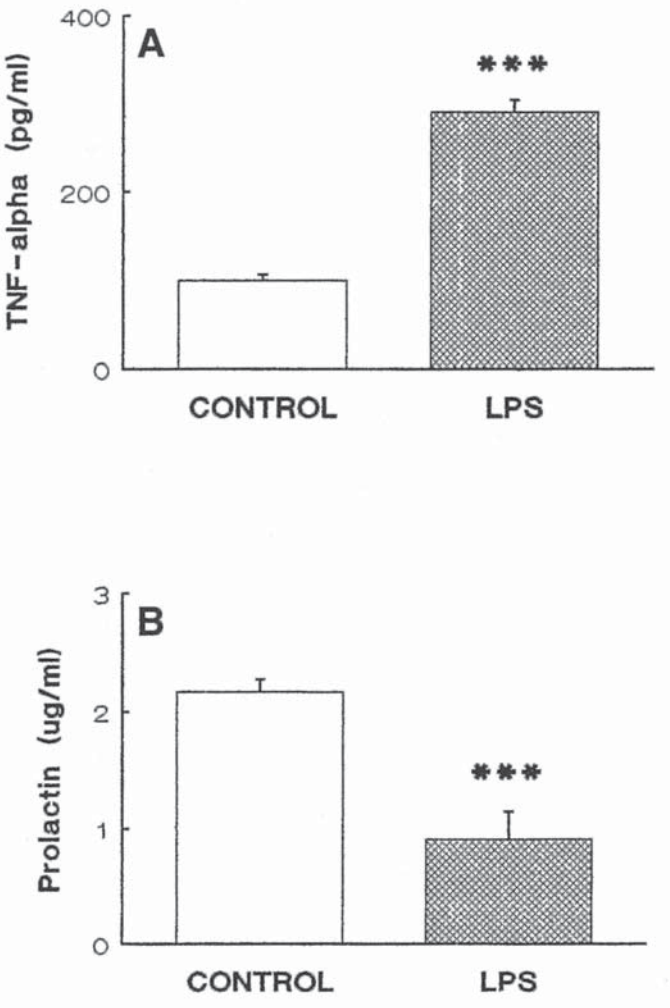


**Fig. 1.** TNF-α **(A)** and prolactin **(B)** release from anterior pituitary cells of lactating rats. Cells from rats on day 5 of lactation were cultured for 3 d in DMEM-S (2.5% FCS) and then incubated with DMEM-S alone or containing LPS (0.1 μg/mL) for 8 h. Each column represents the mean ± SE of 5–8 wells. ***$p < 0.001$ vs control.

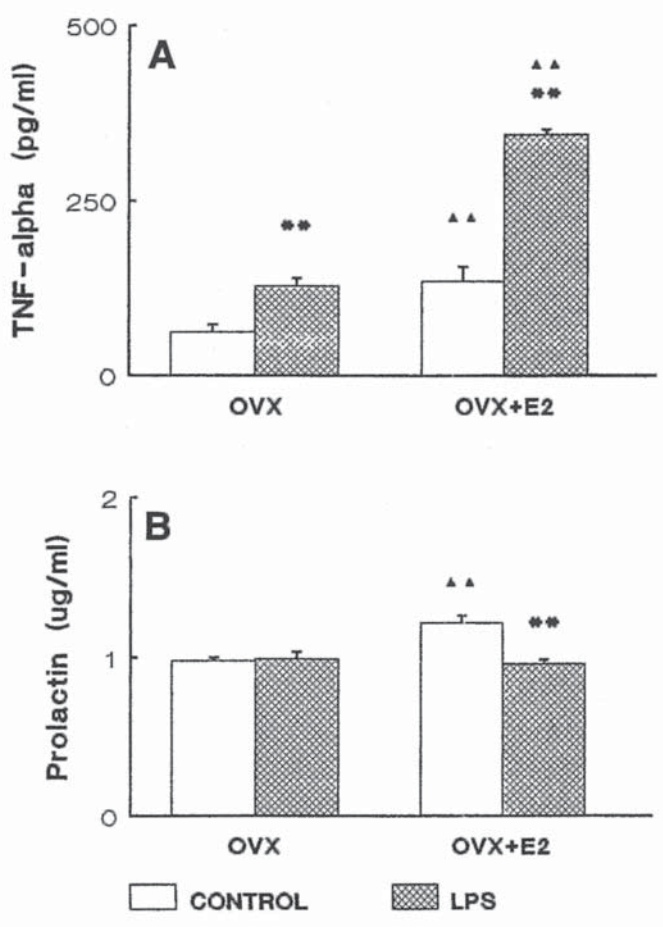


**Fig. 2.** TNF-α **(A)** and prolactin **(B)** release from anterior pituitary cells of chronically estrogenized rats. Cells from OVX and chronically estrogenized OVX rats (OVX + E2) were cultured for 3 d in DMEM-S (2.5% DCC serum) and then incubated with DMEM-S alone or containing LPS (0.1 μg/mL) for 8 h. Each column represents the mean ± SE of 4–7 wells. **$p < 0.01$ vs respective control without LPS. ▲▲$p < 0.01$ vs respective OVX control.

incubated with LPS, TNF-α release showed an approximately fourfold increase. LPS also significantly inhibited prolactin release (Fig. 1).

### *Effect of Chronic Estrogenization on TNF-α and Prolactin Release*

In order to study the involvement of the steroid medium in the effect of LPS, we studied TNF-α release from anterior pituitary cells of chronically estrogenized and ovariectomized (OVX) rats. The basal release of TNF-α was significantly higher in cultures of anterior pituitary cells from chronically estrogenized rats than in those of OVX rats (Fig. 2A). LPS significantly stimulated TNF-α release from anterior pituitary cells of both OVX and chronically estrogenized rats. However, anterior pituitary cells from chronically estrogenized rats released more TNF-α after LPS stimulation than cells from OVX rats. The basal release of prolactin from anterior pituitary cells of chronically estrogenized rats was higher than that of OVX rats (Fig. 2B). The increase in prolactin release induced by estrogens was abrogated by LPS.

### *In Vitro Effect of Estrogens on TNF-α and Prolactin Release*

To investigate whether estrogens could directly affect the pituitary release of TNF-α in response to LPS, cells from OVX rats were incubated in the presence of 17 β-estradiol. The basal release of TNF-α was significantly increased by 17 β-estradiol ($10^{-8}$ *M*) (Fig. 3A). LPS increased TNF-α release from anterior pituitary cells of OVX rats incubated with or without 17 β-estradiol. However, LPS stimulation of TNF-α release was significantly higher in anterior pituitary cells treated with 17 β-estradiol. The basal release of prolactin was significantly higher in the 17 β-estradiol-treated cells (Fig. 3B). LPS had an inhibitory effect on prolactin release from 17 β-estradiol treated cells only.

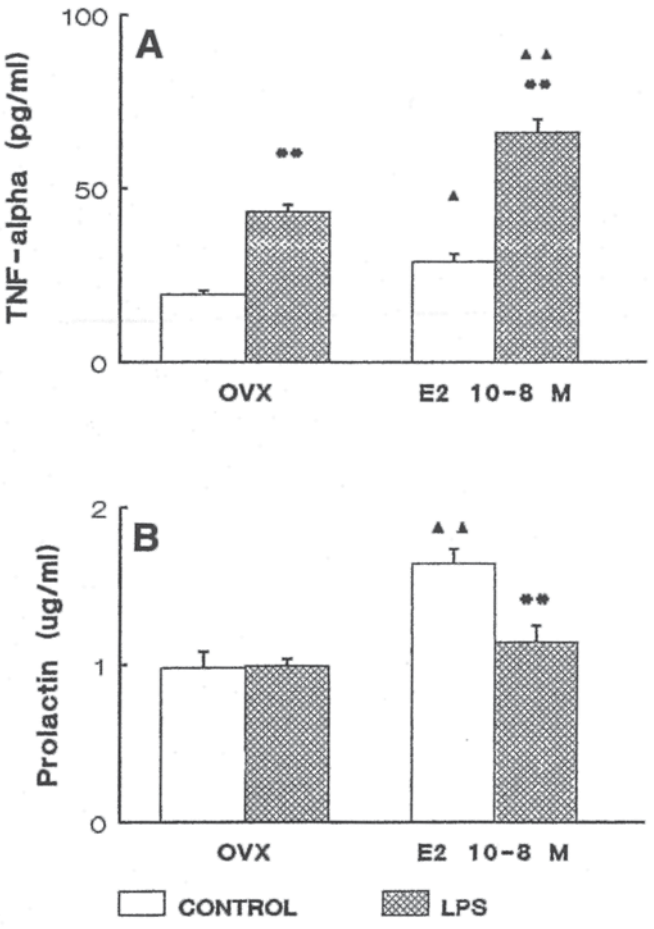


**Fig. 3.** Effect of LPS on TNF-α **(A)** and prolactin **(B)** release from anterior pituitary cells of OVX rats cultured with 17 β-estradiol (E2, $10^{-8}$ *M*). Cells were cultured for 2 d in DMEM-S (2.5% DCC serum) and for another 2 d in the same media with 17 β-estradiol or vehicle. Then, the cells were incubated with DMEM-S alone or containing LPS (0.1 μg/mL) for 8 h. Each column represents the mean ± SE of 5–6 wells. $^{**}p < 0.01$ vs respective control without LPS. ▲$p < 0.05$; ▲▲$p < 0.01$ vs respective OVX control.

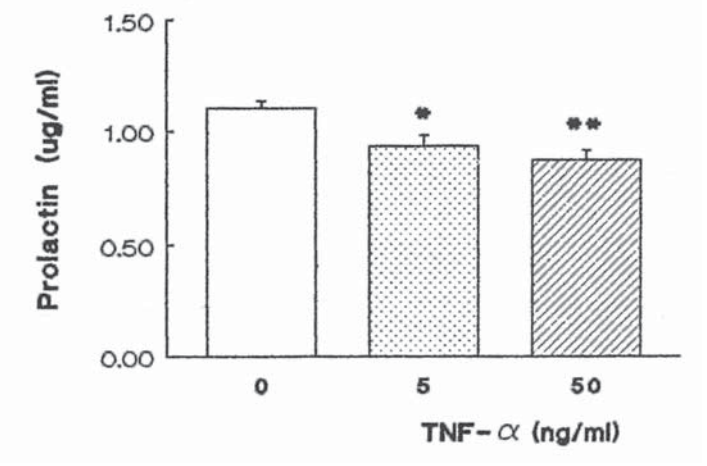


**Fig. 4.** Effect of TNF-α on prolactin release from anterior pituitary cells of rats at random stages of the estral cycle. Cells were cultured for 3 d in DMEM-S (2.5% FCS), kept for 24 h in DMEM-S (0.1% BSA), and then incubated with TNF-α for 8 h. Each column represents the mean ± SE of 10 wells. $^{*}p < 0.05$; $^{**}p < 0.01$ vs control.

### *Effect of TNF-α on Prolactin Release*

To determine whether TNF-α could affect prolactin release, anterior pituitary cells from intact rats were incubated in the presence of TNF-α (5 and 50 ng/mL). TNF-α induced a 15–20% inhibition of prolactin release from anterior pituitary cells (Fig. 4). Under our experimental conditions, TNF-α did not affect cell viability (data not shown).

## Discussion

Evidence has shown that peripheral LPS administration increases TNF-α mRNA in the pituitary gland *(16)*. In the present study, we observed that primary cultures of anterior pituitary cells are able to release TNF-α protein, a product that has not previously been detected in rat anterior pituitary *(31)*. In our experiments, chronic estrogenization increased TNF-α release from anterior pituitary cells, suggesting that TNF-α release may be modulated by the steroid medium. In fact, the presence of 17 β-estradiol in the culture medium stimulated TNF-α release. A similar effect of estrogens seems to occur in the endometrium where ovariectomy abolishes TNF-α expression, an effect reversed by estradiol treatment *(30)*. Moreover, endometrial TNF-α mRNA and protein vary along the estrus cycle *(32)*. Our results also show that LPS directly stimulates TNF-α release from anterior pituitary cells and that estrogens potentiate the stimulatory effect of LPS. As demonstrated elsewhere *(13,14)*, LPS also stimulates IL-6 and IL-1β production by anterior pituitary cells.

The identity of the cells producing TNF-α in the anterior pituitary remains unknown. Since Folliculo Stellate cells are known to produce IL-6 *(33)*, these cells might also be the source of TNF-α in the pituitary. However, other pituitary cells, such as corticotrophs, lactotrophs, and tyrotrophs, also produce some cytokines and growth factors *(12)*, though the possibility that the cells secreting the traditional anterior pituitary hormones could be an alternative site of TNF-α synthesis cannot be discarded.

Our data also show a direct inhibitory effect of LPS on prolactin release from primary cultures of anterior pituitary cells. However, the inhibition of prolactin release was observed only in cultures of cells under the influence of estrogens or from lactating rats, suggesting that estrogens may exert a permissive role on the pituitary response to LPS.

The effect of LPS on prolactin release could be achieved through a direct effect on lactotrophs or by stimulating the synthesis of some cytokines in the pituitary, such as IL-6 or TNF-α. However, it has been reported that IL-6 stimulates prolactin release from cultured anterior pituitary cells *(12)*. On the other hand, TNF-α stimulates prolactin release with

short incubation periods *(17)*, but inhibits this release in long ones *(18,19)*. Under our experimental conditions, TNF-α decreased prolactin release from anterior pituitary cells, suggesting that TNF-α could be involved in the inhibitory effect of LPS on prolactin release. However, the inhibition of prolactin caused by high concentrations of TNF-α is small compared to that induced by LPS. Since LPS could trigger the synthesis and release of several immune mediators in the anterior pituitary, we cannot exclude the possibility that other mediators might contribute to this effect. The combination of several cytokines could act synergistically to suppress prolactin release.

Estrogens are known to stimulate prolactin synthesis and release *(34)* and are involved in the development of prolactin-secreting pituitary tumors *(35)*. It has been suggested that locally produced peptides could interact with hypothalamic hormones and estradiol, whether synergizing or antagonizing their actions, in the anterior pituitary *(12,36)*. Since estrogens potentiate TNF-α release and TNF-α inhibits prolactin release, it could be suggested that pituitary TNF-α could also be a paracrine/autocrine factor controlling anterior pituitary function under the influence of estrogens.

In conclusion, our data show that TNF-α is produced by the pituitary gland; they also raise the possibility that this cytokine may act as a physiological paracrine/autocrine modulator of pituitary function. Pituitary TNF-α could affect pituitary hormone release in response to infection or other pathological conditions.

## Materials and Methods

All drugs, media, and supplements were obtained from Sigma Chemical Co. (St. Louis, MO) except fetal calf serum (GenSa Buenos Aires, Argentina) and rhTNF-α (Promega Co., Madison, WI).

### *Animals*

Adult female Wistar rats were kept under controlled conditions of light (12-h light–dark cycles) and temperature (20–25°C), and were fed with standard lab chow and water ad libitum.

Female rats were ovariectomized under ether anesthesia, and immediately thereafter implanted sc with Silastic capsules containing 0.4 mg of 17 β-estradiol. Control rats were implanted with empty Silastic capsules. Two weeks after surgery, the rats were killed by decapitation.

Lactating rats were kept in individual cages with eight pups. On day 5 postpartum, the mothers were separated from their pups for 60 min and sacrificed.

Intact rats were sacrificed at random stages of the estral cycle.

### *Cell Culture*

Anterior pituitary glands (neurointermediate lobe removed) were obtained within minutes after sacrifice. The tissue was washed several times with Dulbecco's Modified Eagle's Medium (DMEM) and cut into small pieces. Sliced fragments were dispersed enzymatically by successive incubations in DMEM supplemented with 3 mg/mL bovine serum albumin (BSA), containing 5 mg/mL trypsin (Type XII-S from bovine pancreas), 1 mg/mL DNase (Deoxyribonuclease II, Type V from bovine spleen), and 1 mg/mL trypsin inhibitor (Type II-S from soybean), and finally dispersed by extrusion through a Pasteur pipet in Krebs buffer without $Ca^{2+}$ and $Mg^{2+}$. Dispersed cells were washed twice, and suspended in DMEM supplemented (DMEM-S) with 10 µL/mL MEM amino acids, 2.5 µg/mL amphotericin B, 25 µg/mL gentamicin, and 2 m*M* glutamine. Cell viability as assessed by trypan blue exclusion was above 90%. The cells were seeded onto 48-well tissue-culture plates ($12.5 \times 10^4$ cells/0.5 mL/well) and cultured for 3 d in DMEM-S with 2.5% fetal calf serum (FCS) treated with dextran-coated charcoal to remove free steroids (DCC serum). Cells from lactating rats were cultured in DMEM-S with 2.5% FCS. In order to investigate the in vitro effect of estrogens, anterior pituitary cells from OVX rats were dispersed as described above and seeded onto 96-well tissue-culture plates ($8 \times 10^4$ cells/0.25 mL/well). The cells were cultured in DMEM-S with 2.5% DCC serum for 2 d and then for another 2 d in the same medium with 17 β-estradiol $10^{-8}$ *M* or vehicle (2 hydroxypropyl-β-cyclodextrin).

To determine the effect of TNF-α on prolactin release, anterior pituitary cells from rats at random stages of the estral cycle were dispersed and cultured ($8 \times 10^4$ cells/0.25 mL/well) in DMEM-S with 2.5% FCS for 3 d and then in DMEM-S (0.1% BSA) for 24 h.

### *TNF-α and Prolactin Release*

After the culture period, the cells were incubated in DMEM-S alone or containing 0.1 µg/mL of LPS from *Salmonella typhosa* or with TNF-α for 8 h. The media were removed and stored at –70°C until TNF-α determination or at –20°C until assayed for prolactin.

TNF-α was determined by a specific rat TNF-α enzyme immunoassay (Cytoscreen Immunoassay Kit, Biosource, CA). The antibody to rat TNF-α of this assay was shown to crossreact with mouse TNF-α (100%) and human recombinant TNF-α (0.15%). The sensitivity of this assay is <4 pg/mL.

Prolactin was measured by double-antibody radioimmunoassay with reagents provided by the National Hormone and Pituitary Program (Baltimore, MD). The results were expressed in terms of rat prolactin RP-3 standard.

### *Statistical Analysis*

Data were expressed as mean ± SE and evaluated by Student's *t*-test or by analysis of variance (ANOVA) followed by the Student-Newman-Keuls multiple-comparison test. Differences between means were considered significant if $p < 0.05$. All experiments were performed at least twice. Results from individual experiments are presented in the figures.

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