You might find this additional information useful...

This article cites 33 articles, 18 of which you can access free at:
http://ajpendo.physiology.org/cgi/content/full/287/4/E767#BIBL

This article has been cited by 2 other HighWire hosted articles:

Prolonged oestrogen treatment does not correlate with a sustained increase in anterior pituitary mitotic index in ovariectomized Wistar rats
L A Nolan and A Levy
J. Endocrinol., March 1, 2009; 200 (3): 301-309.
[Abstract] [Full Text] [PDF]

Sexual dimorphism of the intracellular heat shock protein 72 response
M. Nickerson, S. L. Kennedy, J. D. Johnson and M. Fleshner
J Appl Physiol, August 1, 2006; 101 (2): 566-575.
[Abstract] [Full Text] [PDF]

Updated information and services including high-resolution figures, can be found at:
http://ajpendo.physiology.org/cgi/content/full/287/4/E767

Additional material and information about *AJP - Endocrinology and Metabolism* can be found at:
http://www.the-aps.org/publications/ajpendo

This information is current as of March 19, 2009 .

Estrogens sensitize anterior pituitary gland to apoptosis

**D. Pisera,[1] M. Candolfi,[1] S. Navarra,[1] J. Ferraris,[1] V. Zaldivar,[1]
G. Jaita,[1] M. G. Castro,[2] and A. Seilicovich[1]**

[1]*Centro de Investigaciones en Reproducción, Facultad de Medicina, Universidad de Buenos Aires,
Buenos Aires C1121ABG, Argentina; and* [2]*Gene Therapeutics Research Institute, Cedars Sinai Medical Center
and David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California 90048*

Pisera, D., M. Candolfi, S. Navarra, J. Ferraris, V. Zaldivar, G. Jaita, M. G. Castro, and A. Seilicovich. Estrogens sensitize anterior pituitary gland to apoptosis. *Am J Physiol Endocrinol Metab* 287: E767–E771, 2004. First published June 1, 2004; 10.1152/ajpendo. 00052.2004.—Tissue homeostasis results from a balance between cell proliferation and cell death by apoptosis. Estradiol affects proliferation as well as apoptosis in hormone-dependent tissues. In the present study, we investigated the apoptotic response of the anterior pituitary gland to lipopolysaccharide (LPS) in cycling female rats, and the influence of estradiol in this response in ovariectomized (OVX) rats. The OVX rats were chronically estrogenized with implanted Silastic capsules containing 1 mg of 17β-estradiol (E2). Cycling or OVX and E2-treated rats were injected with LPS (250 μg/rat ip). Apoptosis was determined by the terminal deoxynucleotidyl-mediated dUTP nick-end labeling (TUNEL) method in sections of the anterior pituitary gland and spleen. Chronic estrogenization induced apoptosis in the anterior pituitary gland. Acute endotoxemia triggered apoptosis of cells in the anterior pituitary gland of E2-treated rats but not of OVX rats. No differences were observed in the apoptotic response to LPS in spleen between OVX and E2-treated rats. The apoptotic response of the anterior pituitary to LPS was variable along the estrous cycle, being higher at proestrus than at estrus or diestrus I. Approximately 75% of the apoptotic cells were identified as lactotropes by immuno-fluorescence. In conclusion, our results indicate that estradiol induces apoptosis and enables the proapoptotic action of LPS in the anterior pituitary gland. Also, our study suggests that estrogens may be involved in anterior pituitary cell renewal during the estrous cycle, sensitizing lactotropes to proapoptotic stimuli.

estrous cycle; estradiol; lactotropes; lipopolysaccharide

LIPOPOLYSACCHARIDE (LPS), an endotoxin of gram-negative bacteria, is commonly used to study neuroendocrine-immune interactions. After systemic administration of LPS, the plasma concentrations of several cytokines, such as TNF-α, IL-1, and IL-6 rise in a temporal-sequential manner (14, 27). Systemic cytokines, as well as those locally released in the central nervous system and pituitary, contribute to the neuroendocrine response to endotoxemia, such as hypothalamic-pituitary-adrenal axis (HPA) activation and hypothalamic-pituitary-gonadal axis inhibition (27, 28, 29). We have reported that systemic LPS and central TNF-α administration exert inhibitory effects on prolactin secretion in male rats by stimulating dopaminergic activity in the hypothalamic-pituitary axis (4), and that LPS (25) and TNF-α (26) reduce in vitro prolactin release from anterior pituitary cells of female rats.

Gonadal steroid hormones modulate the neuroendocrine response to inflammatory stimuli. It has been reported that endotoxin-induced HPA activation is higher in female than in male rats. This sexual difference is abolished by gonadectomy and restored by administration of estradiol to ovariectomized (OVX) rats (28). We have observed that estradiol stimulates basal and LPS-induced TNF-α secretion from anterior pituitary cells of OVX rats (25). Also, TNF-α release from anterior pituitary cells varies along the estrous cycle reaching the highest levels in cells from rats at proestrus (26). The effects of TNF-α in anterior pituitary cells are also modulated by estradiol. We (26) previously reported that TNF-α reduces the proliferation of anterior pituitary cells and inhibits prolactin secretion in an estrogen-dependent manner. TNF-α induces apoptosis of lactotropes and its effect is higher in cells from rats euthanized at proestrus than at other stages of the estrous cycle, suggesting that the steroid environment modulates the proapoptotic action of this cytokine in the anterior pituitary (2). In fact, in anterior pituitary cells from OVX rats, TNF-α significantly increased the percentage of apoptotic lactotropes only when the cells were incubated in the presence of 17β-estradiol (E2) (2).

Considering that LPS induces TNF-α gene expression in the anterior pituitary gland (30), we investigated the effect of the systemic administration of LPS on the apoptotic index in the anterior pituitary of cycling female rats, and the influence of estradiol in the apoptotic response to acute endotoxemia in this gland.

MATERIALS AND METHODS

Animals. Adult female Wistar rats weighing 200–250 g were used. The animals were kept in controlled conditions of light (lights on from 7 AM to 7 PM) and temperature (20–25°C), with water and food available ad libitum. All the experiments were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

In experiments using OVX rats, the animals were castrated under ether anesthesia and implanted under the skin of the back with Silastic capsules (length 20 mm, outer diameter 2 mm) containing 1 mg E2 at the time of ovariectomy. These capsules induce estradiol plasma levels similar to those of rats at proestrus (13). Control animals were implanted with empty capsules. Two weeks later, the animals were injected with LPS (250 μg/rat ip, dissolved in pyrogen-free isotonic saline) or vehicle at 10 AM and euthanized by decapitation 8 h later. In additional experiments, chronically E2-treated rats were injected with LPS (250 μg/rat ip) or vehicle at 10 AM and euthanized 2, 4, 8, or 24 h later.

When cycling female rats were used, animals were monitored by daily vaginal smears. Rats with three or more normal consecutive 4-day estrous cycles were used. Rats at different stages of the estrous cycle were injected with LPS (250 μg/rat ip) or vehicle at 11 AM and euthanized 7 h later.

Drugs. All drugs were obtained from Sigma (St. Louis, MO) including bacterial LPS (*Escherichia Coli* serotype 0111:B8), except terminal deoxynucleotidyltransferase (TdT)-mediated dUTP nick-end labeling (TUNEL) reagents (Roche Molecular Biochemicals, Mannheim, Germany), primary antibodies against anterior pituitary hormones (kindly donated by Dr. A. Parlow, National Hormone and Pituitary Program, Torrance, CA), and anti-guinea pig rhodamine-conjugated secondary antibody (Chemicon International, Temecula, CA).

Determination of anterior pituitary apoptosis by TUNEL method. Pituitary glands were removed within minutes after decapitation. In some experiments spleen was also removed. The tissues were washed in PBS and fixed in 4% formaldehyde and 2% picric acid in 0.1 M PBS, pH 7.4, for 4 h.

Cryostat sections (16 μm) were permeabilized by microwave irradiation in 10 mM citrate buffer, pH 6, for 5 min, and then in the same buffer with 0.1% Triton X-100 for 4 min at room temperature. DNA strand breaks were labeled with digoxigenin-dUTP using TdT (0.5 U/μl) according to the manufacturer's protocol (2). The incorporation of nucleotides into the 3′-OH end of damaged DNA was detected with an antidigoxigenin-fluorescein antibody (1:10). Control slices were incubated without TdT. The sections were mounted in anti-fade mounting medium (DABCO) with 4′,6-diamino-2-phenylindole (DAPI) and observed under fluorescence light microscope (Axiophot; Zeiss, Jena, Germany).

Identification of anterior pituitary cells. Anterior pituitary cell populations undergoing apoptosis were identified in paraffin sections (3 μm) by TUNEL staining combined with indirect immunofluorescence for anterior pituitary hormones. After incubation with digoxigenin-dUTP-TdT, the sections were preincubated with 10% donkey serum in PBS for 60 min and then incubated with primary antibodies against anterior pituitary hormones [guinea pig anti-rat PRL (NHPP-IC, 1:2,000), guinea pig anti-rat GH (NHPP-IC, 1:1,000), guinea pig anti-rat βLH (NHPP-IC-2, 1:1,250) and guinea pig anti-rat adrenocorticotropic hormone (NHPP-IC, 1:500)] in 1% donkey serum in PBS for 60 min. Control sections were incubated with dilution buffer. After being rinsed, the sections were incubated for 60 min with donkey anti-guinea pig rhodamine-conjugated secondary antibody (1:200) plus antidigoxigenin-fluorescein antibody (1:10) in the same buffer. The sections were then washed, mounted, and observed under fluorescence light microscope.

Statistical analysis. TUNEL-positive cells were counted on 10–20 fields (×400) in anterior pituitary sections from each rat. The average of TUNEL-positive cells per field from each rat was considered as an individual value. The averages of TUNEL-positive cells per field were analyzed by two-way ANOVA, followed by Tukey's test. Differences were considered significant when $P < 0.05$. Results are expressed as means ± SE of TUNEL-positive cells per field (×400).

RESULTS

Effect of estradiol on apoptosis induced by LPS in anterior pituitary gland. OVX and chronically E2-treated rats were injected with LPS (250 μg/rat) or vehicle (control) and euthanized 8 h later. The number of TUNEL-positive cells in the neural and intermediate lobes was very scarce and did not allow statistical comparison between experimental groups. Staining with DAPI allowed observation of the nuclear morphological features of apoptosis in cells of the anterior pituitary lobe, such as chromatin condensation and fragmentation (Fig. 1A). Chronic E2 treatment increased the apoptotic index (the

number of TUNEL-positive cells/field) in the anterior pituitary (Fig. 2). LPS administration increased the apoptotic index only in chronically estrogenized rats (Fig. 1*B*, Fig. 2). Approximately 75% of the apoptotic cells were identified as lactotropes by immunofluorescence (Fig. 1*C*). No apoptotic somatotropes, gonadotropes, or corticotropes were observed (not shown).

To evaluate whether the effect of estradiol on the apoptotic response to endotoxemia in the anterior pituitary gland was
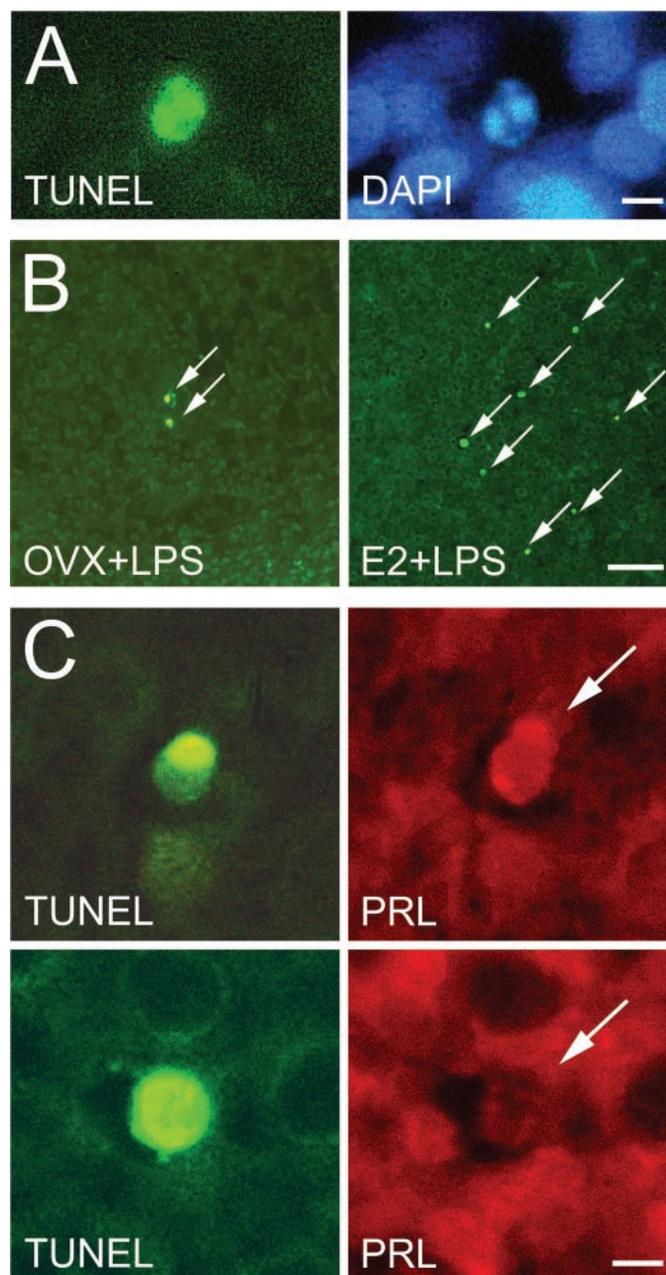

Fig. 1. Apoptotic cells in the anterior pituitary gland. *A*: representative apoptotic anterior pituitary cell showing chromatin fragmentation [*left*, terminal deoxynucleotidyl-mediated dUTP nick-end labeling (TUNEL); *right*, counterstained with DAPI]; scale bar, 5 μm. *B*: representative fields of anterior pituitary sections of ovariectomized (OVX) and 17β-estradiol (E2)-treated rats injected with lipopolysaccharide (LPS). Arrows indicate TUNEL-positive cells; scale bar, 50 μm. *C*: anterior pituitary cells showing DNA fragmentation (*left panels*) and immunofluorescence for prolactin (PRL; *right panels*). Arrows in right panels indicate a TUNEL-positive lactotrope (*top*) and a TUNEL-positive nonidentified anterior pituitary cell (*bottom*); scale bar, 5 μm.
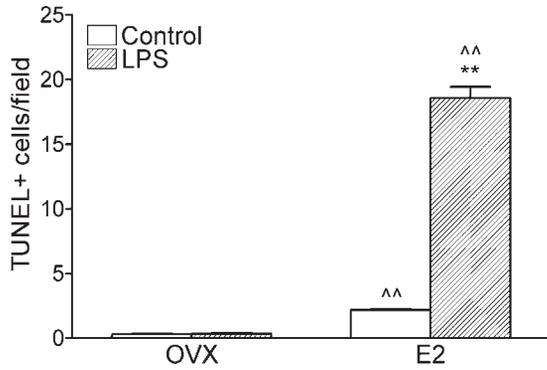
Fig. 2. Chronic E2 treatment to OVX rats induced apoptosis in the anterior pituitary gland and enabled the apoptotic action of endotoxin. OVX and E2-treated rats were intraperitoneally injected with LPS or vehicle and euthanized 8 h later. Each bar represents mean $\pm$ SE of TUNEL-positive cells/field ($\times 400$); $n = 5$ rats per group. The averages of TUNEL-positive cells/field were analyzed by two-way ANOVA, followed by Tukey's test. **$P < 0.01$ vs. respective control without LPS; ^^$P < 0.01$ vs. respective control without estradiol.

Fig. 4. Time course of apoptosis induced by LPS in the anterior pituitary gland of chronically E2-treated rats. E2-treated rats were injected with LPS or vehicle and euthanized at different periods postinjection. Each bar represents mean $\pm$ SE of TUNEL-positive cells/field ($\times 400$); $n = 4–5$ rats per group. The averages of TUNEL-positive cells/field were analyzed by two-way ANOVA, followed by Tukey's test. **$P < 0.01$ vs. respective control without LPS; ^^$P < 0.01$ vs. control rats at other time points.

tissue specific, we explored the effect of LPS on the apoptotic index in spleen of OVX and E2-treated rats. Although the number of TUNEL-positive cells in the spleen was significantly increased by LPS, no differences between OVX and E2-treated rats were observed (Fig. 3).

Time course of apoptosis induced by endotoxemia in the anterior pituitary gland. Chronically E2-treated rats were injected with LPS (250 µg/rat) or vehicle at 10:00 AM and killed at different time periods after endotoxin administration. The apoptotic effect of LPS in the anterior pituitary appeared 4 h postinjection and remained evident at 8 h. At 24 h postinjection the apoptotic index of the anterior pituitary gland returned to basal levels (Fig. 4). In vehicle-treated groups (controls) the number of TUNEL-positive cells varied according to the time period of death posttreatment and was significantly reduced at 8 h postinjection (6 PM) (Fig. 4).

Effect of endotoxemia on apoptosis in anterior pituitary along the estrous cycle. To evaluate the possible modulatory effects of circulating levels of estradiol on the apoptotic action of LPS, female rats at different stages of the estrous cycle were injected with LPS (250 µg/rat) or vehicle at 11 AM and

euthanized 7 h later. The administration of LPS significantly increased the apoptotic index in the anterior pituitary gland of rats at proestrus and diestrus, but not at estrus. The apoptotic response to LPS was significantly higher at proestrus than at diestrus I (Fig. 5).

DISCUSSION

The present study shows that, during acute endotoxemia, apoptosis is triggered in the anterior pituitary gland and that this response is modulated by estradiol. Systemic administration of LPS is a useful tool to elucidate the response of the hypothalamic-pituitary axis to inflammatory stimuli (4, 27, 28, 29). Once LPS binds to circulating LPS-binding protein and CD14, this complex interacts with Toll-like receptor type 4, which can trigger apoptosis (11). Because CD14 and Toll-like receptor type 4 are expressed in the anterior pituitary gland (19), LPS could be exerting a direct proapoptotic effect. However, most of the neuroendocrine actions of LPS are mediated by its stimulatory actions on the secretion of various proinflammatory cytokines in peripheral immune cells, brain, and
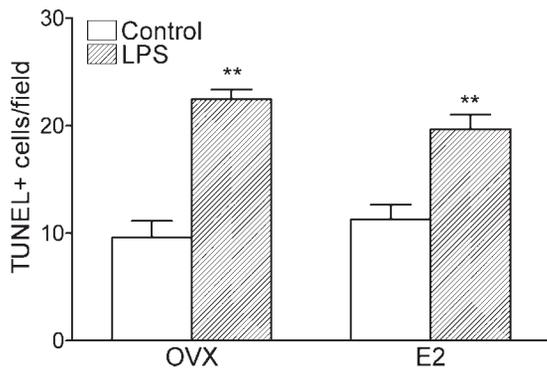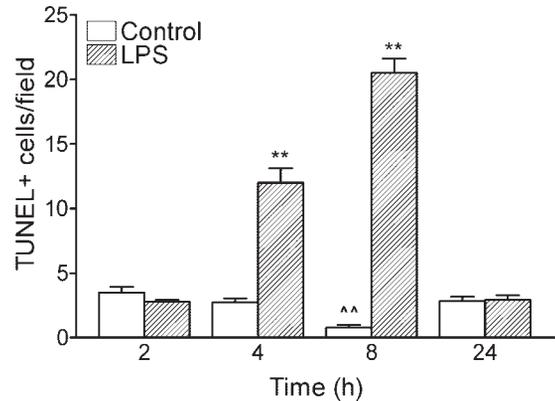
Fig. 3. LPS-induced apoptosis in spleen of OVX and chronically E2-treated rats. OVX and E2-treated rats were injected with LPS or vehicle and euthanized 8 h later. Each bar represents mean $\pm$ SE of TUNEL-positive cells/field ($\times 400$); $n = 5$ rats per group. The averages of TUNEL-positive cells/field were analyzed by two-way ANOVA. **$P < 0.01$ vs. respective control without LPS.
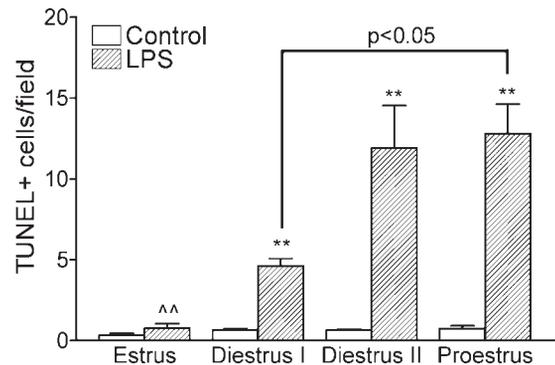
Fig. 5. The proapoptotic effect of LPS in the anterior pituitary gland was variable along the estrous cycle. Cycling female rats were injected with LPS or vehicle at 11 AM and were then euthanized 7 h later. Each column represents mean $\pm$ SE of TUNEL-positive cells/field ($\times 400$); $n = 4–6$ rats per group. The averages of TUNEL-positive cells/field were analyzed by two-way ANOVA, followed by Tukey's test. **$P < 0.01$ vs. respective control without LPS, ^^$P < 0.01$ vs. LPS-treated rats at other stages of estrous cycle.

pituitary gland (9, 27, 28, 29, 30). TNF-α plays a central role during endotoxemia, initiating a cascade of synthesis of other factors required for an appropriate neuroendocrine response to infection (14). TNF-α is expressed in the pituitary gland after LPS administration (30) and is released from anterior pituitary cells in response to LPS (25). Since the anterior pituitary gland expresses TNF-α receptors (31) and this cytokine induces apoptosis of anterior pituitary cells (2), circulating and/or locally produced TNF-α could be involved in the apoptotic response of the anterior pituitary to endotoxemia.

The immune response presents sexual dimorphism. Autoimmune diseases are more prevalent in females than in males, and the development of some autoimmune diseases is modulated by circulating levels of gonadal steroids (8, 20). In postmenopausal women, estrogens inhibit cytokine release and HPA activation in response to inflammatory stimuli (23). However, in OVX rats, estradiol enhances the adrenocorticotropic homone secretory response to LPS, without affecting the increase in TNF-α, IL-1, or IL-6 plasma levels (28). In the present study, we observed that the systemic administration of LPS induces apoptosis of anterior pituitary gland in chronically estrogenized rats but not in OVX rats. These results suggest that estradiol increases the responsiveness of anterior pituitary cells to LPS, which by direct action or through auto/paracrine mediators may trigger apoptosis in the anterior pituitary gland. The lack of effect of LPS in OVX rats seems to be tissue specific, because no differences were observed in LPS-induced apoptosis in the spleen between OVX and E2-treated rats. Chronic estradiol treatment not only sensitized the anterior pituitary cells to the proapoptotic effect of LPS, but also induced apoptosis per se in the anterior pituitary gland. The variations observed in basal anterior pituitary apoptosis in E2-treated rats killed at different times suggest diurnal changes in the proapoptotic action of estradiol, as was described for the effects of estrogens on lactotrope proliferation (13).

Estradiol has been reported to enhance neuronal survival through genomic and nongenomic mechanisms (10), and to inhibit the apoptosis induced by TNF-α in neurons (16). Also, estrogens have been shown to exert proliferative and antiapoptotic actions in a prolactin secreting pituitary cell line derived from F344 rats, a strain particularly sensitive to estradiol-induced pituitary tumors (3). However, estradiol inhibits IGF-I- and insulin-induced anterior pituitary cell proliferation (15), and induces apoptosis in somatolactotrope GH_4C_1 cells that overexpress ER-α (17). Evidence shows that lactotrope apoptosis occurring after termination of lactation involves changes in the expression of modulators of cell survival such as Bax and p53 (1). The p53 tumor suppressor protein can induce cell death, and therefore, the inhibition of p53-induced apoptosis can promote tumor progression (7). Estradiol increases p53 gene expression in human breast cancer MCF-7 cells, and it has been suggested that this effect may contribute to regulatory pathways leading to inhibition of estradiol-induced cell proliferation (24). In fact, in Sprague-Dawley rats, anterior pituitary p53 mRNA levels increase in response to estrogen treatment, whereas they remain undetectable in F344 rats (34). There is evidence that cell proliferation and apoptosis are coupled processes. The activation of cell proliferation necessarily primes the cellular apoptotic program that, unless abrogated by appropriate survival signals, automatically removes the affected cell (5). In fact, it was observed that adrenalec-

tomy, followed by glucocorticoid replacement induces linked mitotic and apoptotic activities in the anterior pituitary, with apoptotic sensitivity confined to cells that have previously entered the cell cycle (18, 22).

We observed ~75% of the TUNEL-positive cells in the anterior pituitary gland of estrogenized rats treated with LPS were lactotropes. Although we could not detect apoptosis in somatotropes, gonadotropes or corticotropes we cannot rule out that LPS may induce apoptosis in other cell types of the anterior pituitary. It was suggested that rapid degradation of secretory vesicles makes it difficult to identify which cell types in this gland undergo programmed cell death (21, 33). Hence, it is possible that any secretory cell at late stages of apoptosis, including lactotropes, could account for nonidentified apoptotic cells.

Lactotropes are the anterior pituitary cell subpopulation with the highest cell turnover during the estrous cycle. They exhibit a cyclic pattern of cell proliferation, reaching a peak in the morning of estrus (12). Our previous findings indicate that estradiol sensitizes lactotropes to the proapoptotic effect of TNF-α, and that this action of TNF-α is highest in anterior pituitary cells from rats killed at proestrus (2). Thus we hypothesized that lactotropes are renewed in each estrous cycle by cell proliferation during estrus and cell death by apoptosis during proestrus (2). In fact, Yin and Arita (33) have reported that anterior pituitary cells undergo cyclic changes in apoptosis throughout the estrous cycle, with the highest levels in the morning of proestrus. In our experimental conditions, no significant changes in the basal apoptotic index in the anterior pituitary gland were observed along the estrous cycle, probably because rats were euthanized in the evening of proestrus. Some evidence suggests that estrogens may be involved in anterior pituitary apoptosis during the estrous cycle. First, TNF-α-induced apoptosis in anterior pituitary cells is predominant in cells from rats at proestrus and is estrogen dependent (2). Second, the apoptotic response of the anterior pituitary gland to LPS is higher in rats at proestrus than at other stages of the estrous cycle and also estrogen dependent. Because circulating levels of estrogens are higher at proestrus (6), our findings suggest that endogenous variations in circulating gonadal steroids modulate apoptosis in the anterior pituitary. This effect of estradiol could be mediated by modifying cell phenotypic features that increase the responsiveness of the anterior pituitary cells to proapoptotic signals. Also, it has been suggested that the sensitizing action of estradiol is required for lactotrope proliferation at estrus (32). Therefore, estradiol may participate in anterior pituitary cell renewal sensitizing the cells to both mitogenic and apoptotic signals.

In conclusion, estradiol induces apoptosis in the anterior pituitary gland and increases its sensitivity to proapoptotic stimuli such as LPS, suggesting that estrogens may be involved in anterior pituitary apoptosis during the estrous cycle.

REFERENCES

1. **Ahlbom E, Grandison L, Zhivotovsky B, and Ceccatelli S.** Termination of lactation induces apoptosis and alters the expression of the Bcl-2 family members in the rat anterior pituitary. *Endocrinology* 139: 2465–2471, 1998.

2. **Candolfi M, Zaldivar V, De Laurentiis A, Jaita G, Pisera D, and Seilicovich A.** TNF-α induces apoptosis of lactotropes from female rats. *Endocrinology* 143: 3611–3617, 2002.

3. **Caporali S, Imai M, Altucci L, Cancemi M, Caristi S, Cicatiello L, Matarese F, Penta R, Sarkar DK, Bresciani F, and Weisz A.** Distinct signaling pathways mediate stimulation of cell cycle progression and prevention of apoptotic cell death by estrogen in rat pituitary tumor PR1 cells. *Mol Biol Cell* 14: 5051–5059, 2003.

4. **De Laurentiis A, Pisera D, Caruso C, Candolfi M, Mohn C, Rettori V, and Seilicovich A.** Lipopolysaccharide- and tumor necrosis factor-α-induced changes in prolactin secretion and dopaminergic activity in the hypothalamic-pituitary axis. *Neuroimmunomodulation* 10: 30–39, 2002.

5. **Evan G and Littlewood T.** A matter of life and cell death. *Science* 281: 1317–1322, 1998.

6. **Freeman ME.** The neuroendocrine control of the ovarian cycle of the rat. In: *The Physiology of Reproduction* (2nd ed.), edited by Knobil E and Neill JD. New York: Raven, 1994, chapt. 46, p. 613–658.

7. **Fridman JS and Lowe SW.** Control of apoptosis by p53. *Oncogene* 22: 9030–9040, 2003.

8. **Gaillard RC and Spinedi E.** Sex- and Stress-steroids interactions and the immune system: evidence for a neuroendocrine-immunological sexual dimorphism. *Domest Anim Endocrinol* 15: 345–352, 1998.

9. **Gloddek J, Lohrer P, Stalla J, Arzt E, Stalla GK, and Renner U.** The intrapituitary stimulatory effect of lipopolysaccharide on ACTH secretion is mediated by paracrine-acting IL-6. *Exp Clin Endocrinol Diabetes* 109: 410–415, 2001.

10. **Green PS, Simpkins JW.** Neuroprotective effects of estrogens: potential mechanisms of action. *Int J Dev Neurosci* 18: 347–358, 2000.

11. **Haase R, Kirschning CJ, Sing A, Schrottner P, Fukase K, Kusumoto S, Wagner H, Heesemann J, and Ruckdeschel K.** A dominant role of Toll-like receptor 4 in the signaling of apoptosis in bacteria-faced macrophages. *J Immunol* 171: 4294–4303, 2003.

12. **Hashi A, Mazawa S, Kato J, and Arita J.** Pentobarbital anesthesia during the proestrous afternoon blocks lactotroph proliferation occurring on estrus in female rats. *Endocrinology* 136: 4665–4671, 1995.

13. **Hashi A, Mazawa S, Chen SY, Yamakawa K, Kato J, and Arita J.** Estradiol-induced diurnal changes in lactotroph proliferation and their hypothalamic regulation in ovariectomized rats. *Endocrinology* 137: 3246–3252, 1996.

14. **Kakizaki Y, Watanobe H, Kohsaka A, and Suda T.** Temporal profiles of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in the plasma and hypothalamic paraventricular nucleus after intravenous or intraperitoneal administration of lipopolysaccharide in the rat: estimation by push-pull perfusion. *Endocr J* 46: 487–496, 1999.

15. **Kawashima K, Yamakawa K, Takahashi W, Takizawa S, Yin P, Sugiyama N, Kanba S, and Arita J.** The estrogen-occupied estrogen receptor functions as a negative regulator to inhibit cell proliferation induced by insulin/IGF-1: a cell context-specific antimitogenic action of estradiol on rat lactotrophs in culture. *Endocrinology* 143: 2750–2758, 2002.

16. **Koski CL, Hila S, and Hoffman GE.** Regulation of cytokine-induced neuron death by ovarian hormones: involvement of antiapoptotic protein expression and c-JUN N-terminal kinase-mediated proapoptotic signaling. *Endocrinology* 145: 95–103, 2004.

17. **Lee EJ, Duan WR, Jakacka M, Gehm BD, and Jameson JL.** Dominant negative ER induces apoptosis in GH₄ pituitary lactotrope cells and inhibits tumor growth in nude mice. *Endocrinology* 142: 3756–3763, 2001.

18. **Levy A.** Physiological implications of pituitary trophic activity. *J Endocrinol* 174: 147–155, 2002.

19. **Lohrer P, Gloddek J, Nagashima AC, Korali Z, Hopfner U, Pereda MP, Arzt E, Stalla GK, and Renner U.** Lipopolysaccharide directly stimulates the intrapituitary interleukin-6 production by folliculostellate cells via specific receptors and the p38alpha mitogen-activated protein kinase/nuclear factor-kappaB pathway. *Endocrinology* 141: 4457–4465, 2000.

20. **McMurray RW.** Estrogen, prolactin, and autoimmunity: actions and interactions. *Int Immunopharmacol* 1: 995–1008, 2001.

21. **Nolan LA, Kavanagh E, Lightman SL, and Levy A.** Anterior pituitary cell population control: basal cell turnover and the effects of adrenalectomy and dexamethasone treatment. *J Neuroendocrinol* 10: 207–215, 1998.

22. **Nolan LA and Levy A.** Temporally sensitive trophic responsiveness of the adrenalectomized rat anterior pituitary to dexamethasone challenge: relationship between mitotic activity and apoptotic sensitivity. *Endocrinology* 144: 212–219, 2003.

23. **Pfeilschifter J, Köditz R, Pfohl M, and Schatz H.** Changes in proinflammatory cytokine activity after menopause. *Endocr Rev* 23: 90–119, 2002.

24. **Qin C, Nguyen T, Stewart J, Samudio I, Burghardt R, and Safe S.** Estrogen up-regulation of p53 gene expression in MCF-7 breast cancer cells is mediated by calmodulin kinase IV-dependent activation of a nuclear factor kappaB/CCAAT-binding transcription factor-1 complex. *Mol Endocrinol* 16: 1793–1809, 2002.

25. **Theas MS, De Laurentiis A, Lasaga M, Pisera D, Duvilanski B, and Seilicovich A.** Effect of lipopolysaccharide on tumor necrosis factor and prolactin secretion from rat anterior pituitary cells. *Endocrine* 8: 241–245, 1998.

26. **Theas S, Pisera D, Duvilanski B, De Laurentiis A, Pampillo M, Lasaga M, and Seilicovich A.** Estrogens modulate the inhibitory effect of tumor necrosis factor-α on anterior pituitary growth and prolactin release. *Endocrine* 12: 249–255, 2000.

27. **Turnbull A and Rivier C.** Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev* 79: 1–70, 1999.

28. **Watanobe H and Yoneda M.** A mechanism underlying the sexually dimorphic ACTH response to lipopolysaccharide in rats: sex steroid modulation of cytokine binding sites in the hypothalamus. *J Physiol* 547: 221–232, 2003.

29. **Watanobe H and Hayakawa Y.** Hypothalamic interleukin-1β and tumor necrosis factor-α, but not interleukin-6, mediate the endotoxin-induced suppression of the reproductive axis in rats. *Endocrinology* 144: 4868–4875, 2003.

30. **Whiteside MB, Quan N, and Herkenharn M.** Induction of pituitary cytokine transcripts by peripheral lipopolysaccharide. *J Neuroendocrinol* 11: 115–120, 1999.

31. **Wolvers DA, Marquette C, Berkenbosch F, and Haour F.** Tumor necrosis factor-alpha: specific binding sites in rodent brain and pituitary gland. *Eur Cytokine Netw* 4: 377–381, 1993.

32. **Yin P, Kawashima K, and Arita J.** Direct actions of estradiol on the anterior pituitary gland are required for hypothalamus-dependent lactotrope proliferation and secretory surges of luteinizing hormone but not of prolactin in female rats. *Neuroendocrinology* 75: 392–401, 2002.

33. **Yin P and Arita J.** Proestrus surge of gonadotropin-releasing hormone secretion inhibits apoptosis of anterior pituitary cells in cycling female rats. *Neuroendocrinology* 76: 272–282, 2002.

34. **Ying C.** Potential involvement of tumor suppressor gene expression in the formation of estrogen-inducible pituitary tumors in rats. *Endocr J* 47: 1–5, 2000.