Estrogens exert a rapid apoptotic action in anterior pituitary cells


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Zárate S, Jaita G, Zaldivar V, Radl DB, Eijo G, Ferraris J, Pisera D, Seilicovich A. Estrogens exert a rapid apoptotic action in anterior pituitary cells. Am J Physiol Endocrinol Metab 296: E664–E671, 2009. First published January 21, 2009; doi:10.1152/ajpendo.90785.2008.—It is now accepted that estrogens not only stimulate lactotrope proliferation but also sensitize anterior pituitary cells to proapoptotic stimuli. In addition to their classical mechanism of action through binding to intracellular estrogen receptors (ERs), there is increasing evidence that estrogens exert rapid actions mediated by cell membrane-localized ERs (mERs). In the present study, we examined the involvement of membrane-initiated steroid signaling in the proapoptotic action of estradiol in primary cultures of anterior pituitary cells from ovariectomized rats by using estren, a synthetic estrogen with no effect on classical transcription and a cell-impermeable 17β-estradiol conjugate (E2-BSA). Both compounds induced cell death of anterior pituitary cells after 60 min of incubation as assessed by flow cytometry and the [3-(4,5-dimethylthiazol-2-yl)]-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay. Estren, E2, and E2-BSA induced apoptosis of lactotropes and somatotropes as evaluated by the deoxynucleotidyltransferase-mediated dUTP nick end-labeling assay and immunodetection of lactotrope antigens (PRL) and growth hormone (GH). The proapoptotic effect of E2-BSA was abrogated by 182,780, an antagonist of ERs. The expression of membrane-associated ERα was observed in PRL- and GH-bearing cells. Our results indicate that estradiol is able to exert a rapid apoptotic action in anterior pituitary cells, especially lactotropes and somatotropes, by a mechanism triggered by mERs. This mechanism could be involved in anterior pituitary cell turnover.

estrogen; membrane receptors; pituitary; lactotropes; somatotropes; apoptosis

ESTROGENS ARE INVOLVED in a wide range of both physiological and pathological processes, such as regulation of the reproductive cycle and pregnancy (57), neuroprotection (3), tumor necrosis factor-α (TNF-α) and FasL-induced apoptosis of anterior pituitary cells, especially lactotropes and somatotropes (5), which occurs soon after estrogen exposure and involves the rapid activation of many signaling molecules, such as insulin-like growth factor and epidermal growth factor receptors, p21 RAS and RAF, MAPK1/3, protein kinase B, protein kinase C, calcium channel, nitric oxide, and Maxi-K channels (26, 51). The activation of many of these pathways can, in turn, modulate the activity of transcription factors, thereby influencing downstream gene transcription (1). They can also interact with other cytoplasmic proteins and regulate their function (47).

In the rat, anterior pituitary cells follow a cyclic pattern of proliferation and death during the estrous cycle (17), in which lactotropes are the cells with the highest turnover (38). Although estradiol induces hyperprolactinemia and lactotrope proliferation (14), the rate of anterior pituitary cell apoptosis has a peak at proestrous (59), when estrogen levels are the highest. Previous studies from our laboratory have shown that estrogens sensitize the anterior pituitary gland to the proapoptotic effect of endotoxin, with this action being higher at proestrus than at any other stage of the estrous cycle (41). Also, tumor necrosis factor-α- and FasL-induced apoptosis of anterior pituitary cells is predominant at proestrus and estrogen dependent (6, 21). Moreover, dopamine has been reported to elicit a proapoptotic action on anterior pituitary cells in an estrogen-dependent manner (42). 17β-Estradiol (E2) has also been shown to exert a direct proapoptotic action on somatotropes (7).

Rapid effects of estrogens, such as stimulation of prolactin (PRL) release, have been reported in the anterior pituitary. These effects are incompatible with genomic regulation and suggest that this steroid is able to initiate a noncanonical pathway in this gland (4, 5, 11).

Considering that the changes in hormone levels occur in a rapid fashion along the estrous cycle and that estradiol participates in both mitotic and apoptotic events in anterior pituitary cells, we examined the involvement of membrane-initiated steroid signaling in the proapoptotic action of estradiol in anterior pituitary cells, especially lactotropes and somatotropes. To achieve this aim, we used 4-estren-3α-17β-diol (estren), a synthetic estrogen that exerts extranuclear, kinase-initiated actions and has minimal effects on classical transcription (1), and E2 bound to bovine serum albumin, which is a

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nondiffusible form of E₂ and thereby renders the steroid active only at the cell surface (53).

**MATERIALS AND METHODS**

**Drugs.** All drugs and reagents were obtained from Sigma Chemical (St. Louis, MO) except for phenol red-free Dulbecco’s modified Eagle’s medium and supplements (D-MEM; Gibco, Invitrogen, Carlsbad, CA), FBS (GIBCO, Buenos Aires, Argentina), all terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling (TUNEL) reagents (Roche Molecular Biochemicals, Mannheim, Germany), primary antibodies against anterior pituitary hormones (Dr. A. Parlow, National Hormone and Pituitary Program, Torrance, CA), anti-guinea pig rhodamine-conjugated secondary antibody (Chemicon International, Temecula, CA), and the materials indicated below.

**Animals.** Adult female Wistar rats (200–250 g) were kept in controlled conditions of light (12:12-h light-dark cycles) and temperature (20–25°C). Rats were fed standard laboratory chow and water ad libitum and kept in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animal protocols were previously approved by the Ethics Committee of the School of Medicine, University of Buenos Aires. The rats were ovariectomized (OVX) 2 wk before the experiments under ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip) anesthesia. Anterior pituitary glands were removed within minutes after decapitation.

**Cell culture.** A pool of anterior pituitary cells from 3–8 OVX rats were used for each culture. Anterior pituitary glands were washed several times with D-MEM supplemented with 10 μM minimal essential medium amino acids, 2 mM glutamine, 5.6 μg/ml amphotericin B, 25 μg/ml gentamicin (DMEM-S), and 3 mg/ml BSA. The glands were then cut into small fragments. Sliced fragments were dispersed enzymatically by successive incubations in DMEM-S-BSA, containing 0.75% trypsin, 10% FBS previously treated with 0.025% dextran-0.25% charcoal (FBS-DCC) to remove steroids, and 45 μg/ml deoxyribonuclease type I (Invitrogen). Finally, the cells were dispersed by extrusion through a Pasteur pipette in Krebs buffer without Ca²⁺ and Mg²⁺. Dispersed cells were washed and resuspended in DMEM-S with 10% FBS-DCC. Cell viability as assessed by trypan blue exclusion was >90%. Anterior pituitary cells were seeded on coverslides in 24-well tissue culture plates (1 × 10⁵ cells·well⁻¹) for the TUNEL assay, on 24-well tissue culture plates (3 × 10⁵  cells·well⁻¹·well⁻¹) for flow cytometry analysis [fluorescence-activated cell sorter (FACS) analysis], and on 96-well tissue culture plates (5 × 10⁴ cells·well⁻¹·well⁻¹) for determination of metabolic activity of viable cells [(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS assay)]. After 1 h at 37°C, the OD was read at a wavelength of 490 nm. The quantity of formazan product is directly proportional to the number of living cells in culture.

**Preparation of E₂-BSA devoid of free E₂.** Nonpermeant conjugates of E₂ have been reported to be devoid of free E₂, as assayed by radioimmunoassay (52). Both untreated and treated E₂-BSA induced apoptosis of anterior pituitary cells, with no difference in the effect of the two preparations of the conjugate, as assessed by annexin-V-fluorescein isothiocyanate (FITC) (BD Pharmingen, San Jose, CA) and propidium iodide (PI) staining and FACS (data not shown). All of the following experiments were performed using this conjugate devoid of free E₂ carefully prepared before each experiment.

**Assessment of the metabolic activity of viable cells.** The metabolic activity of viable cells was determined by the MTS assay (Promega, Madison, WI). Reaction solution (20 μl) containing MTS (final concentration 333 μg/ml) and an electron coupling reagent (phenazinethosulfate, final concentration 25 μM) were added to each well containing 100 μl of culture medium. After 4 h at 37°C, the OD was read at a wavelength of 490 nm. The quantity of formazan product is directly proportional to the number of living cells in culture.

**Detection of apoptosis by flow cytometry.** Cells were harvested with 0.025% trypsin-EDTA (GIBCO, Invitrogen), washed with cold PBS, and resuspended in binding buffer (10 mM HEPES/NaOH, pH 7.40, 140 mM NaCl, and 2.5 mM CaCl₂). Next, the cells were incubated with 5 μl annexin-V-FITC and 10 μl PI (50 μg/ml) for 15 min at room temperature in the dark. Cells were immediately analyzed by flow cytometry with a FACScan (Becton Dickinson). Annexin-V-positive/PI-negative cells were considered early apoptotic, whereas annexin-V-negative/PI-positive cells were considered necrotic cells. Double-positive (annexin-V positive/PI positive) cells were considered to be in a late stage of apoptosis (40). Analysis of apoptotic anterior pituitary cells was performed using WinMDI 98 software.

**Microscopic determination of DNA fragmentation by TUNEL.** To identify the populations within the anterior pituitary gland that undergo apoptosis, we used the TUNEL assay. After the culture period, cells were fixed with 4% formaldehyde in PBS for 10 min and permeabilized by microwave irradiation. DNA strand breaks were labeled with digoxigenin-deoxyuridine triphosphate using terminal deoxynucleotidyl transferase (0.18 μg/ml) according to the manufacturer’s protocol. After incubation with 10% normal donkey serum and 10% normal sheep serum in PBS for 40 min, the cells were incubated for 1 h with guinea pig rat PRL antiserum (1:1,500) or guinea pig rat growth hormone (GH) antiserum (1:2,000). Next, the slides were incubated with antidigoxigenin-fluorescein antibody (1:10) to detect incorporation of nucleotides in the 3' OH end of damaged DNA and rhodamine-conjugated antigen guinea pig secondary antibody (1:200) in the same buffer. Slides were mounted with mounting medium for fluorescence ( Vectashield, Vector Laboratories, Burlingame, CA) containing 4',6-diamidino-2-phenylindole (DAPI) for DNA staining and visualized in a fluorescence light microscope (Axiphot; Carl Zeiss, Jena, Germany). The percentage of apoptotic anterior pituitary cells was calculated as [(TUNEL⁺/total cells) × 100, the percentage of apoptotic lactotropes as [(TUNEL⁺/PRL⁺/total PRL⁺ cells) × 100, and the percentage of apoptotic somatotropes as [(TUNEL⁺/GH⁺/total GH⁺ cells) × 100.

**Expression of membrane estrogen receptor α (mERα) in anterior pituitary cells, lactotropes, and somatotropes.** Anterior pituitary cells from adult intact female Wistar rats were dispersed as described above for cell culture. Dispersed cells were washed, resuspended in 0.1% PBS-BSA and separated into tubes at a density of 5 × 10⁶ cells/tube. Next, the cells were incubated for 1 h at 37°C to stabilize cell membranes. Next, the cells were fixed with 0.1% paraformaldehyde in PBS for 10 min at room temperature in the dark and permeabilized by a 10-min incubation with PBS-0.05% saponin (MB Biomedicals). The cells were incubated for 1 h with guinea pig rat PRL antiserum (1:2,000) or guinea pig rat GH antiserum (1:2,000) in PBS-0.05% saponin followed by a 40-min incubation with a FITC-conjugated antigen guinea pig secondary antibody for flow cytometry analysis (1:150; Chemicon International) or a rhodamine-conjugated antigen guinea pig secondary antibody for confocal microscopy (1:150; Chemicon International) in the same buffer. Because cell permeabilization with
saponin is reversible (32), the cells were washed, resuspended in PBS-0.1% BSA, and rocked for 1 h at 37°C to allow repair of cell membranes. Next, the cells were incubated for 1 h with rabbit antirat ERα antibody MC-20 (3 μg/10^6 cells; Santa Cruz Biotechnology, Santa Cruz, CA) in PBS followed by a 40-min incubation with a phycoerithrin-conjugated antirabbit secondary antibody for flow cytometry analysis (1:67; Vector Laboratories) or a FITC-conjugated antirabbit secondary antibody for confocal microscopy (1:67; Vector Laboratories) in the same buffer. For isotype controls, the cells were incubated with guinea pig serum instead of PRL or GH antiserum and rabbit immunoglobulin G instead of ERα antibody. The cells were washed, resuspended in PBS, and analyzed by flow cytometry using a FACScan (Becton-Dickinson). There was no difference in the percentage of mERα-positive cells in anterior pituitary cells fixed and labeled vs. those that went through the entire process of fixation, permeabilization, repair, and labeling (data not shown). Data analysis was performed using WinMDI 98 software. The percentage of mERα-positive total anterior pituitary cells, calculated by adding the percentages of mERα-positive cells that were either positive or negative for PRL or GH, was expressed as [(mERα+/total cells) × 100. The percentage of mERα-positive lactotropes was expressed as [(mERα+ PRL+)/total PRL+ cells] × 100 and the percentage of mERα-positive somatotropes as [(mERα+ GH+)/total GH+ cells] × 100. Aliquots of the cell suspensions incubated with the corresponding antibodies were smeared on glass slides and mounted in Vectashield (Vector Laboratories) for confocal and phase-contrast microscopy (FV300; Olympus, Tokyo, Japan). The photographs were acquired using Fluoview version 3.3 software.

Statistical analysis. Cell viability data (from MTS assay) and percentage of apoptotic cells (by FACS) were expressed as means ± SE of 8 wells from 2 independent experiments. *P < 0.05 vs. control (1-way ANOVA followed by Dunnett’s test).
cell apoptosis with that of E2. Both steroids increased the time tested (Fig. 1).

min of incubation, and this effect was also observed at a longer incubation, as tested by TUNEL (Fig. 2).

Next, we compared the effect of estren on anterior pituitary cell apoptosis with that of E2. Both steroids increased the percentage of apoptotic anterior pituitary cells after 120 min of incubation, as tested by TUNEL (Fig. 2A). The basal apoptotic levels detected by TUNEL were lower than those determined by annexin-V/PI staining and FACS, since the TUNEL method only shows the apoptotic cells that remain attached to the slides, whereas FACS includes all of the apoptotic cells, both the attached cells plus the floating ones. Indirect immunofluorescence assays for GH and PRL were performed to identify the attached cells plus the floating ones. Indirect immunofluorescence assays for GH and PRL were performed to identify GH- and PRL-bearing cells, double indirect immunofluorescence assays for both ERα and either PRL or GH were performed.

Membrane-initiated proapoptotic action of E2. To explore whether E2 triggers a signaling cascade at the level of the cell membrane leading to proapoptotic events in anterior pituitary cells, we used E2-BSA, a membrane-impermeant conjugate of E2. To characterize the actions of E2-BSA on anterior pituitary cell viability in terms of incubation time, we evaluated the cytotoxic effect of this conjugate by MTS assay. A significant decrease in the metabolic activity of viable cells was evident after 60 min of incubation and continued up to the last period tested (Fig. 3A). Next, the effect of different concentrations of E2-BSA on the apoptosis of anterior pituitary cells was evaluated by annexin-V/PI staining and FACS. This conjugate induced apoptosis of these cells at concentrations of $10^{-10}$ M or higher (Fig. 3B).

Next, we compared the effect of E2 and E2-BSA on the apoptosis of anterior pituitary cells. When the cells were incubated with either E2 or E2-BSA, a significant increase in the percentage of apoptotic cells was observed as assessed by both annexin-V/PI staining and TUNEL (Fig. 4, A and B). To determine whether E2-BSA has a proapoptotic effect on different anterior pituitary populations, we evaluated the percentage of TUNEL-positive somatotropes and lactotropes. E2-BSA significantly induced apoptosis of these two anterior pituitary cell populations (Fig. 4, C and D).

To examine the specificity of estrogen action on the apoptosis of anterior pituitary cells, we studied the effect of the pure antagonist of ERs ICI-182,780. ICI-182,780 completely blocked E2-BSA-induced apoptosis of anterior pituitary cells (Fig. 5A). To further investigate the mechanism by which E2 exerts its proapoptotic action on anterior pituitary cells, we determined the effect of E2-BSA in the presence of an inhibitor of caspases, Z-VAD-FMK. Z-VAD-FMK reversed E2-BSA-induced apoptosis of anterior pituitary cells (Fig. 5B).

Expression of membrane ERs on anterior pituitary cells, lactotropes, and somatotropes. To study the expression of ERα on the membrane of anterior pituitary cells, especially in PRL- and GH-bearing cells, double indirect immunofluorescence assays for both ERα and either PRL or GH were performed. We could detect the presence of ERα on the membrane of both

Fig. 4. Effect of E2-BSA and E2 on the percentage of apoptosis (A) and TUNEL-positive anterior pituitary cells (B), somatotropes (C), and lactotropes (D). Cells were incubated with vehicle, E2 ($10^{-9}$ M), or E2-BSA ($10^{-9}$ M) for 120 min, and the percentage of apoptotic cells was analyzed by annexin-V/PI staining and FACS (A) or TUNEL assay (B–D). A: each bar represents the mean ± SE of 3 wells from 1 experiment representative of 2 independent experiments. **$P < 0.01$ vs. vehicle (1-way ANOVA followed by Dunnett’s test). B–D: each bar represents the percentage ± CL of TUNEL-positive anterior pituitary cells (B), somatotropes (C), and lactotropes (D) ($n = 300–3,500$ cells from 4 separate experiments). *$P < 0.05$ and **$P < 0.01$ vs. vehicle ($t$ test).
ion flux and hormone release (5, 11). It has been reported that both E2-BSA and E2 induce a rapid release of PRL from anterior pituitary fragments from both male and female rats and in anterior pituitary cell cultures from female rats (11, 16). In the pituitary tumor cell line GH3/B6/P10, which expresses the membrane receptor ERα, both E2 and E2-peroxidase, a cell-impermeable analog of E2, induce a rapid, concentration-dependent increase in intracellular calcium levels and PRL release (5). PRL release is also affected by ERα-specific antibodies, which are too large to diffuse into cells (37), indicating the presence of an active membrane ER (mER) that can transduce signals initiated at the plasma membrane. In fact, it has been reported that the antimitogenic effect of estradiol in lactotropes involves membrane-associated ERs (16). In our study, we could detect apoptotic events after a short incubation of anterior pituitary cells with E2-BSA. This result suggests that the death signaling cascade is initiated by this steroid very quickly, an action probably mediated by mERs that modulate signal transduction pathways in the cytoplasm and thus may affect gene expression only indirectly (26, 51).

mERs have been described in many tissues, where they seem to play a crucial role in alternative mechanisms of estrogen action (18, 26, 30, 51). The identity of mERs is still a matter of controversy (26). mERs have been described both in normal anterior pituitary cells, where they show a patchy distribution on the surface of the cells (11), and in the pituitary tumor cell line GH3/B6, in which the mER has been identified as a membrane form of the classical ERα (55). A membrane form of the classical ERα but not of ERβ in lactotropes has also been reported recently (16). In the present study, we detected a mER that might be a classical ERα associated to the plasma membrane or other ER with a similar amino acid composition and a close structural similarity (55). Interestingly, in the present study, we show that 40% of lactotropes, the anterior pituitary cells with the highest turnover, express mER. In addition to ERα and ERβ, several ER variant forms have been described in many tissues, although in the pituitary they are expressed at very low levels (48). The adult female rat pituitary also expresses a unique COOH-terminal truncated form of ERα, TERP-1, whose expression reaches a peak at proestrus and is estrogen-dependent (48). TERP-1 cannot bind DNA but has been reported to regulate some estrogenic effects through protein-protein interactions. TERP-1 has been found in both the cytoplasm and the nucleus of anterior pituitary cells (43) but, to our knowledge, its presence at or near the plasma membrane has not been reported. Because the antibody against ERβ used in our study binds to the COOH-terminal region of the receptor, the resulting mERα detected could involve the full-length ERα and/or TERP-1.

In the present study, the proapoptotic effect of E2-BSA was observed in lactotropes and somatotropes, two anterior pituitary cell populations highly related to each other developmentally, suggesting the presence of membrane-initiated estrogenic effects in both cell types. The anterior pituitary expresses primarily ERα, although low levels of ERβ have been described in nearly every adult pituitary cell type (48). Mitchner et al. (29) reported that ERα and ERβ are expressed in varying proportions in the lactotrope, corticotrope, and gonadotrope secretory populations of the anterior pituitary gland. About 50% of lactotropes express ERα, whereas <30% express ERβ (29). In normal human pituitary slices, ERα mRNA was
Localized in ~3% of somatotropes (15). To our knowledge, ERα has not been detected in GH-producing cells in the rat pituitary. However, direct effects of E2 on GH secretion have been reported in the MtT/S somatotrope cell line (20). Moreover, estrogen increases the percentage of GH mRNA and GH-expressing anterior pituitary cells from female rats at diestrus (10). The currently accepted model for somatotrope-lactotrope lineage development indicates that the bihormonal mammosomatotropes are obligatory intermediary cells in the development of lactotropes (2). In fact, the presence of ERs, among other factors, is responsible for the expression of PRL in mammosomatotrope cells (2). More recently, an alternative model for somatotrope-lactotrope lineage development suggested that most somatotropes arise primarily through the lactotrope lineage via the mammosomatotropes (27). Because we did not dual-label secretory cells, we cannot rule out the possibility that at least part of the somatotropes that die by estrogen-induced apoptosis are in fact bihormonal mammosomatotropes, or even multihormonal cells that may express ERs (9). Our results show the presence of ERα in ~12% of GH-bearing cells, which may involve a mixed population of these three types of GH-producing cells.

Our study shows that estren also exerts a rapid proapoptotic action in anterior pituitary cells. Some authors have reported the lack of typical genomic effects of estren, which do not seem to stimulate transcription via the induction of direct binding of ER to estrogen response element sites on DNA (24, 25). Although estren has been reported to be an ER modulator with transcriptional activity, this effect seems to need concentrations of at least three orders of magnitude higher than that of E2 and a longer incubation period (31, 25). In our experiments, only a 100-fold higher concentration of estren was used, compared with E2, on the basis of its lower binding affinity for ER (24). Estrogen and androgens have been suggested to have opposing roles in mediating trophic responses in the anterior pituitary, with the former, either exogenous or generated by local aromatization of testosterone, stimulating anterior pituitary mitotic activity while the latter facilitating the inhibition of anterior pituitary mitotic activity induced by gonadectomy (36). Estren is able to bind and activate both ERs and the androgen receptor (1); thus, the summative effects of mitosis along with apoptosis in anterior pituitary cells in the long term must be taken into account. In the present study, however, both E2 and estren induce a similar increase in the percentage of apoptotic cells, even when cells were incubated with the steroids for 24 h (data not shown). Further studies should be performed to define the mechanism by which estren exerts its proapoptotic action on anterior pituitary cells.

Cell proliferation and cell death are two processes tightly linked. There is evidence showing that activation of the cell proliferation machinery necessarily prepares the cellular apoptotic program that, unless revoked by proper survival signals,
automatically removes the affected cell (12). Indeed, it has been reported that both adrenalectomy and gonadectomy followed by dexamethasone or testosterone treatment induce an increase in coupled processes of mitosis and apoptosis in the anterior pituitary (35, 36). A delicate balance between cell survival and cell death governed by ER is now well recognized (22). It has been proposed that the E2-ER complex interprets its natural environment to decide cell survival or death (39). In addition to the well-known proliferative effects of estrogens on lactotropes, recent evidence has shown that estrogens also trigger antiproliferative responses in anterior pituitary cells (19, 23) and sensitize them to apoptotic signals (8, 16, 58).

It has been reported that, under basal conditions, the normal young adult male pituitary gland has a rate of parenchymal cellular turnover of ~1.5%/day (34), whereas the anterior pituitary mitotic activity in cycling female rats doubles that in males overall, with a peak of mitosis occurring in estrus (38). Lactotropes have shown to be the population showing the maximal mitotic activity in this stage of the estrous cycle (17). These facts indicate that even small changes in the number of mitotic and apoptotic cells that may seem unremarkable could have a considerable physiological impact (34).

The mER has been reported to be short-lived and more tightly regulated than its nuclear counterpart, allowing for a very dynamic control of its levels (56). In pituitary cells, the presence of mERs would allow them to rapidly and accurately assess changes in hormonal levels and respond to them either by inducing proliferation or death. In this way, pituitary cells and especially lactotropes could change their sensitivity to proapoptotic or mitotic stimuli depending on the pattern of circulating gonadal hormones, as occurs during the estrous cycle.

In summary, our results show that estradiol exerts a rapid apoptotic response in anterior pituitary cells, probably mediated through membrane-associated ERs, suggesting that estrogens may play a role in the maintenance of cell number in the anterior pituitary gland. In anterior pituitary cells, which are exposed to cyclic changing levels of estrogens during the estrous cycle, the activation of mERs could be a mechanism to rapidly respond to the changing environment.

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