Endocr (2011) 39:21–27
DOI 10.1007/s12020-010-9407-4

ORIGINAL ARTICLE

Gonadal steroids modulate Fas-induced apoptosis of lactotropes and somatotropes

Gabriela Jaita · Sandra Zárate · Luciana Ferrari · Daniela Radl · Jimena Ferraris · Guadalupe Eijo · Verónica Zaldivar · Daniel Pisera · Adriana Seilicovich

Received: 4 June 2010 / Accepted: 16 September 2010 / Published online: 11 November 2010

Abstract We have previously reported that Fas activation induces apoptosis of anterior pituitary cells from rats at proestrus but not at diestrus and in an estrogen-dependent manner. In this study, we evaluated the effect of Fas activation on apoptosis of lactotropes and somatotropes during the estrous cycle and explored the action of gonadal steroids on Fas-induced apoptosis. Also, we studied whether changes in Fas expression are involved in the apoptotic response of anterior pituitary cells. Fas activation increased the percentage of TUNEL-positive lactotropes and somatotropes at proestrus but not at diestrus. FasL triggered apoptosis of somatotropes only when cells from ovariectomized rats were cultured in the presence of 17 β-estradiol (E2). Progesterone (P4) blocked the apoptotic action of the Fas/FasL system in lactotropes and somatotropes incubated with E2. Both E2 and P4 increased the percentage of cells expressing Fas at the cell membrane. Our results show that Fas activation induces apoptosis of lactotropes and somatotropes at proestrus but not at diestrus. Gonadal steroids may be involved in the apoptotic response of lactotropes and somatotropes, suggesting that Fas activation is implicated in the renewal of these pituitary subpopulations during the estrous cycle. The effect of gonadal steroids on Fas expression may be only partially involved in regulation of the Fas/FasL apoptotic pathway in the anterior pituitary gland.

G. Jaita · S. Zárate · L. Ferrari · D. Radl · J. Ferraris · G. Eijo · V. Zaldivar · D. Pisera · A. Seilicovich (✉)
Facultad de Medicina, Instituto de Investigaciones en Reproducción, Universidad de Buenos Aires, Paraguay 2155, piso 10, ABG1121 Buenos Aires, Argentina
e-mail: adyseili@fmed.uba.ar

Keywords Fas · Lactotropes · Somatotropes · Estrogens · Progesterone · Apoptosis

Introduction

The Fas/FasL system is one of the main apoptotic pathways involved in maintenance of tissue homeostasis in several organs [1–5]. Both FasL and Fas are transmembrane proteins belonging to the TNF-α/TNFRI family [6]. Interaction between Fas receptor and FasL, its physiologic ligand, is followed by the assembly of several intracellular proteins. One is the Fas-associated death domain protein (FADD) which leads to auto-activation of initiator caspases such as caspase 8 and 10. Once activated, they can stimulate other downstream caspases that execute cell death by apoptosis [6]. The Fas/FasL system has been implicated not only in apoptosis but also in anti-apoptotic and proliferative processes [6, 7].

Expression of the Fas/FasL system was initially considered to be restricted to lymphoid tissues. However, it has been detected in non-lymphoid tissues such as brain, placenta, ovary and bone, among others [8–12]. We have localized Fas and FasL in anterior pituitary cells, mainly in lactotropes and somatotropes. The percentage of Fas and FasL immunoreactive cells is higher in anterior pituitary cells from rats at proestrus than at diestrus. Also, 17 β-estradiol increases the percentage of anterior pituitary cells from ovariectomized rats expressing either Fas or FasL [13].

The anterior pituitary gland undergoes a process of cell renewal during the estrous cycle of the female rat [14]. Cyclic changes in circulating levels of gonadal steroids are involved in the modulation of anterior pituitary cell turnover [15–18]. Plasma levels of estradiol reach a peak value in the morning of proestrus, whereas systemic levels of progesterone show a high peak in the evening of proestrus

and a lower one on the first day of diestrus [19]. Lactotropes are the anterior pituitary cell subpopulation with the highest rate of proliferation followed by somatotropes, corticotropes, and gonadotropes [15]. During the estrous cycle, lactotropes account for almost all the proliferation activity detected at estrus in the anterior pituitary [15] whereas the highest rate of apoptosis of anterior pituitary cells is observed at proestrus [18, 20].

Several reports suggest that gonadal steroids affect cell renewal and tissue homeostasis in hormone-dependent tissues such as ovary, endometrium, and the mammary gland [1, 3, 5, 21]. This action of gonadal steroids may be achieved through modulation of Fas/FasL expression and function [1, 3, 5]. We previously showed that Fas activation triggers apoptosis of anterior pituitary cells from rats killed at proestrus but not at diestrus. Fas-mediated apoptosis of anterior pituitary cells is modulated by estradiol, suggesting that the Fas/FasL system may represent a hormone-sensitive pathway in the control of cell renewal in this gland [13].

This study was undertaken to determine the role of the Fas/FasL system in the turnover of lactotropes and somatotropes during the estrous cycle and the influence of gonadal steroids on Fas-induced apoptosis of these pituitary subpopulations. We also explored whether changes in Fas expression are involved in the apoptotic response of anterior pituitary cells.

Results

Fas-induced apoptosis of lactotropes and somatotropes is modulated by gonadal steroids

Considering that Fas and FasL expression is mainly distributed among lactotropes and somatotropes [13], we explored whether the Fas/FasL system is involved in cell turnover of these subpopulations during the estrous cycle. We studied the effect of Fas activation on apoptosis of lactotropes and somatotropes from cycling rats killed at proestrus or diestrus. Activation of Fas increased the percentage of apoptotic lactotropes and somatotropes from rats killed at proestrus but not at diestrus (Fig. 1a, b).

We previously reported that the Fas/FasL system exerts a proapoptotic effect on total anterior pituitary cells and

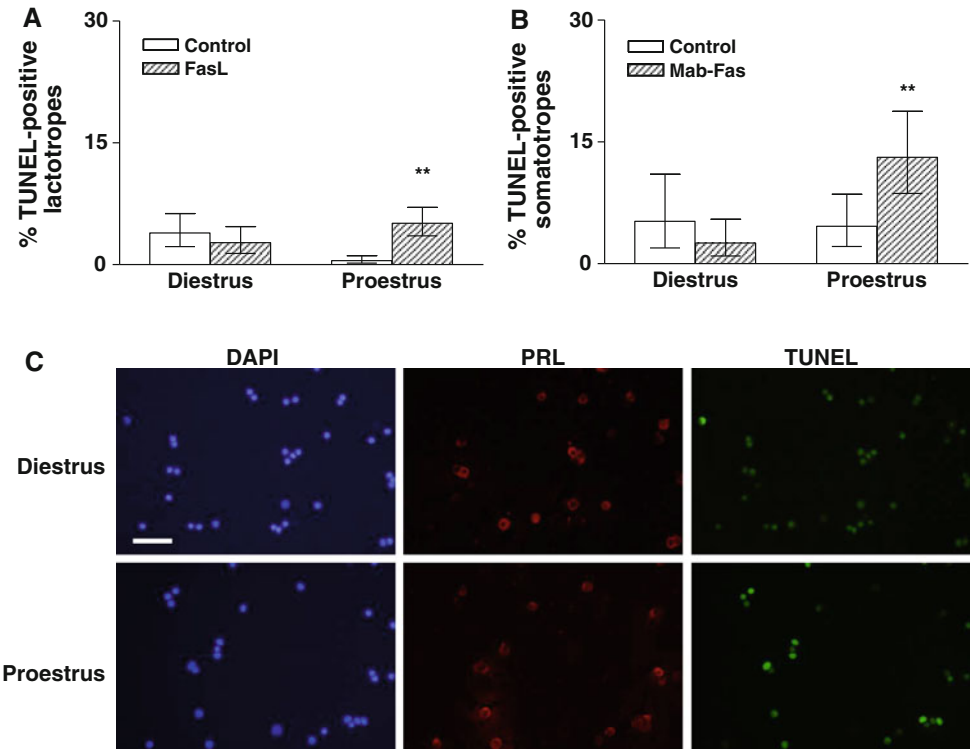

Fig. 1 Effect of Fas activation on percentage of apoptotic lactotropes (**a**) and somatotropes (**b**) from rats killed at selected stages of the estrous cycle. Anterior pituitary cells from rats killed at diestrus or proestrus were incubated with an anti-Fas antibody (Mab–Fas 1 μg/ml) or recombinant ligand (FasL 10 ng/ml) for 24 h. **a** Each column represents the percentage ± CL of TUNEL-positive lactotropes. $n = 390$–440 lactotropes/group from two independent experiments. $^{**}P < 0.01$ versus respective control without FasL. χ^2 test. **b** Each column represents the percentage ± CL of TUNEL-positive somatotropes. $n = 115$–240 somatotropes/group from two independent experiments. $^{**}P < 0.01$ versus respective control without Mab–Fas. χ^2 test. **c** Representative microphotograph of TUNEL-positive lactotropes from rats killed at diestrus or proestrus incubated with FasL. PRL-bearing cells were identified by indirect immunofluorescence. *Scale bar* 50 μm

lactotropes in an estrogen-dependent manner [13]. To evaluate whether estradiol influences the apoptotic action of the Fas/FasL system in somatotropes, we determined the effect of FasL (10 ng/ml) on the percentage of TUNEL-positive somatotropes from OVX rats incubated with 17 β-estradiol (E2, 10^{-9} M). Fas activation triggered apoptosis of somatotropes only when cells were cultured in the presence of E2 (Fig. 2). We have shown that progesterone per se does not modify the apoptotic rate of anterior pituitary cells but blocks the permissive action of estradiol on TNF-α-induced apoptosis of both lactotropes and somatotropes [22]. In order to evaluate whether progesterone modulates the apoptotic response of anterior pituitary cells to Fas activation, we explored the effect of this steroid on Fas-induced apoptosis of lactotropes and somatotropes incubated in the presence of E2. In the absence of gonadal steroids, Fas activation did not induce apoptosis of either lactotropes or somatotropes (data not shown). FasL-induced apoptosis of lactotropes and somatotropes cultured with 17 β-estradiol was blocked by progesterone (P4, 10^{-6} M) (Fig. 3a, b).

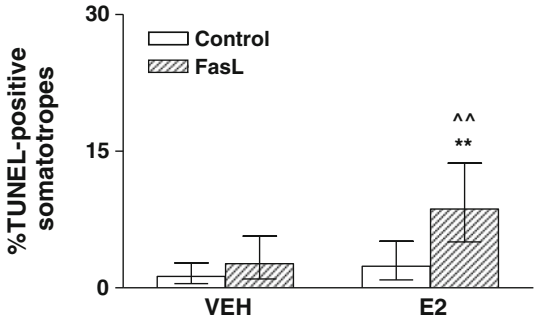

Fig. 2 Fas-induced apoptosis of somatotropes. Anterior pituitary cells from OVX rats were incubated with FasL (10 ng/ml) in the presence of ethanol (VEH 1 µl/l) or 17 β-estradiol (E2 10^{-9} M) for 24 h. Each column represents the percentage ± CL of TUNEL-positive somatotropes. $n = 200$–300 somatotropes/group from two independent experiments. ** $P < 0.01$ versus respective control without FasL, ^^$P < 0.01$ versus respective control without E2. χ^2 test

Gonadal steroids modify Fas expression in the anterior pituitary

In order to study whether changes in Fas expression are involved in the apoptotic response to Fas activation we evaluated the influence of gonadal steroids on Fas expression in anterior pituitary cells from OVX rats. Neither E2 nor P4 modified the percentage of cells expressing total Fas protein (Fig. 4a). However, both gonadal steroids increased the percentage of cells expressing Fas receptor in the cell membrane, without showing additive effects (Fig. 4b).

Discussion

Fluctuation of gonadal steroids in each estrous cycle could turn on or off several cell pathways to allow tissue remodeling. During the estrous cycle, the changing pattern of gonadal steroids modulates both cell proliferation and death in the anterior pituitary gland, thus maintaining tissue homeostasis [14]. In the anterior pituitary gland of female rats, lactotropes present the highest proliferation rate followed by somatotropes [15]. It has been shown that estrogens are not exclusively a mitotic stimulus to PRL-bearing cells but are also capable of stimulating division in several other pituitary cellular subpopulations in the anterior pituitary gland of male rats [23]. Our study shows that Fas activation induces apoptosis of both lactotropes and somatotropes from rats at proestrus when circulating estradiol levels are highest, but not at diestrus. We have reported that estradiol sensitizes lactotropes to the proapoptotic action of Fas activation [13]. Now we show that

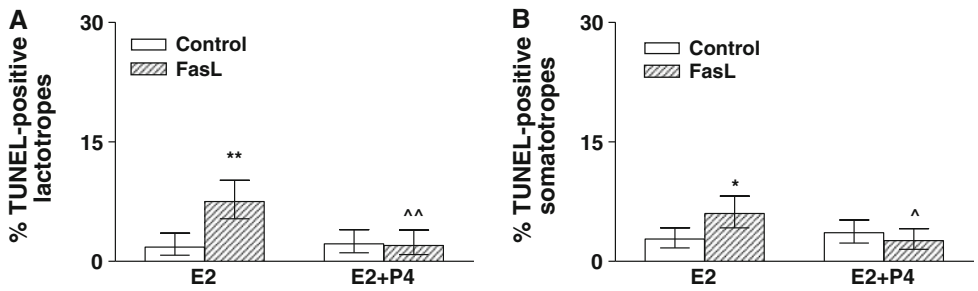

Fig. 3 Effect of progesterone on apoptotic action of the Fas/FasL system in lactotropes (**a**) and somatotropes (**b**). Anterior pituitary cells from OVX rats were incubated with FasL (10 ng/ml) in the presence of 17 β-estradiol (E2, 10^{-9} M) alone or with progesterone (P4, 10^{-6} M) for 24 h. **a** Each column represents the percentage ± CL of TUNEL-positive lactotropes. $n = 350$–450 lactotropes/group from three independent experiments. **b** Each column represents the percentage ± CL of TUNEL-positive somatotropes. $n = 600$–750 somatotropes/group from six independent experiments. *$P < 0.05$, **$P < 0.01$ versus respective control without FasL, ^$P < 0.05$, ^^$P < 0.01$ versus respective control without P4. χ^2 test

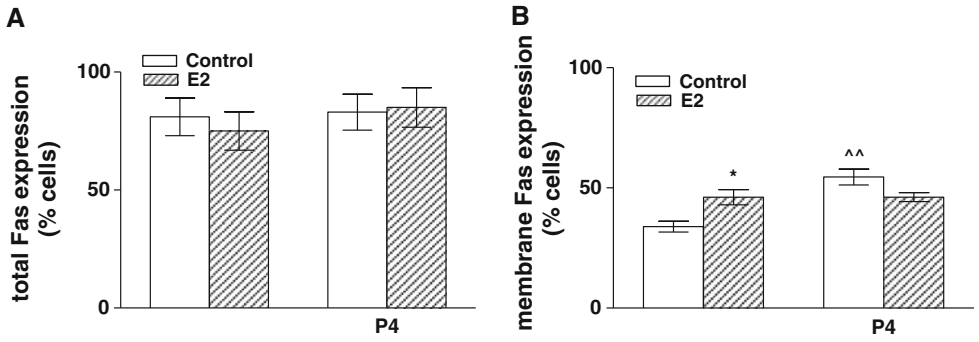

Fig. 4 Effects of gonadal steroids on Fas expression in anterior pituitary cells. Anterior pituitary cells from OVX rats were incubated with ethanol (control, 10 µl/l), 17 β-estradiol (E2, 10^{-9} M), and/or progesterone (P4, 10^{-6} M). (**a**) Total Fas receptor was determined by FACS. Each column represents the percentage ± SE of cells expressing total Fas in each condition from three independent experiments. (**b**) Membrane-localized Fas receptor (mFas) was determined by FACS. Each column represents the percentage ± SE of cells expressing mFas in each condition from five independent experiments.*$P < 0.05$ versus respective control without E2, ^^$P < 0.01$ versus respective control without P4. Two-way ANOVA

Fas activation also induces apoptosis of somatotropes only when estradiol is present. Therefore, sensitivity of lactotropes and somatotropes to Fas activation at proestrus may be due to high circulating levels of estrogens during this stage of the estrous cycle. In contrast to the classical model of development of pituitary cell types [24], it has been postulated that somatotropes originate from a lactotrope lineage which goes through an intermediate somatolactotrope stage [25]. As we did not dual-label secretory cells we cannot rule out that the action of Fas/FasL system on GH and PRL-bearing cells can be exerted on somatolactotropes. The lack of apoptotic response of lactotropes and somatotropes to Fas activation at diestrus could be related to the peak of progesterone present at this stage of the estrous cycle. In fact, our results show that progesterone interferes with the permissive action of estradiol in the proapoptotic effect of Fas activation on lactotropes and somatotropes. Similarly, progesterone was reported to impair the sensitizing effect of estrogens in TNF-α-induced apoptosis of these pituitary subpopulations [22]. This inhibitory effect of progesterone was suggested to be exerted through progesterone receptors [22]. Since gonadotropes seem to be the only subpopulation in the rat anterior pituitary gland that expresses progesterone receptors [26], it is possible to speculate that the inhibitory effect of progesterone on Fas-induced apoptosis could result from paracrine action. In the morning of proestrus, there is an expansion in the GH-bearing cell subpopulation which also expresses LH and FSH [27]. Therefore, the inhibitory effect of progesterone on Fas-induced apoptosis of somatotropes could be exerted on the somatogonadotrope subpopulation. Since the female rat anterior pituitary has more mitosis at estrus and more apoptosis at proestrus [15, 18, 20], it is possible to speculate that in females, lactotrope proliferation at estrus prepares this pituitary subpopulation for the requirements of the proestrus prolactin surge and prolactin secretion during pregnancy. When pregnancy does not occur, lactotropes may die at the following proestrus to reduce the increased number of these cells. Since the Fas/FasL system triggers apoptosis in this subpopulation at proestrus, Fas activation could be a mechanism involved in limiting the expansion of lactotropes in the absence of pregnancy. GH secretion shows a variable pattern along the estrous cycle. Serum GH was reported to peak near the preovulatory gonadotropin surge [28]. Also, GH mRNA expression peaks at proestrus [27]. Hence, the participation of the Fas/FasL system on the renewal of somatotropes during the estrous cycle would provide an additional mechanism in the regulation of GH.

The increase in the expression of Fas and FasL in the anterior pituitary gland may be one of the mechanisms involved in the estrogen-modulated cell renewal. The percentage of cells expressing total Fas protein did not vary with E2 and/or P4 treatments. Our previous immunocytochemical data [13] show that the percentage of anterior pituitary cells expressing Fas in the cell membrane is increased by estrogen action. Our present data also show that P4 increases the percentage of cells expressing Fas in the cell membrane suggesting that both gonadal steroids may increase Fas translocation to cell membrane. Fas stimulation triggers an endocytic pathway and recycling endosomes deliver Fas receptor to the cell surface [29]. Fas-associated tyrosine phosphatase (FAP-1) inhibits Fas export to the cell membrane [30]. Considering that antiestrogens were reported to increase FAP-1 expression it is possible to suggest that estrogens may increase the translocation of Fas to the cell membrane through an effect on FAP-1 expression [31]. Since progesterone also increases Fas expression in the cell membrane, the inhibitory effect of this steroid on the permissive action of estradiol on Fas-induced apoptosis of anterior pituitary cells may not be related to availability of Fas at the cell membrane.

In summary, our findings show that the Fas/FasL system triggers apoptosis of lactotropes and somatotropes at a specific stage of the estrous cycle suggesting that Fas activation is involved in the renewal of both pituitary subpopulations during the estrous cycle. The presence of Fas is not enough to induce apoptosis in anterior pituitary gland suggesting that, as occurs in other hormone-dependent tissues, gonadal steroids in addition to regulating Fas expression may also modulate other steps of Fas apoptotic pathway. The Fas/FasL system could be one of several physiologic mechanisms that control cyclic tissue remodeling in the anterior pituitary gland in response to cyclic changes in levels of gonadal steroids during the estrous cycle. Since the mechanisms involved in apoptosis are tightly regulated, alterations in their function could have considerable physiopathological consequences. Comprehension of the modulatory role of gonadal steroids in apoptotic pathways in the anterior pituitary could contribute to understand basic mechanisms implicated in the pathogenesis of pituitary tumors and perhaps to develop novel strategies in their treatment.

Materials and methods

Drugs

All drugs, media, and supplements were obtained from Gibco, Invitrogen (Carlsbad, CA, USA) except for Dulbecco's Modified Eagle's Medium, bovine serum albumin, and EDTA (Sigma Chemical Co. St. Louis, MO, USA), fetal bovine serum (GenSa, Buenos Aires, Argentina), membrane bound Fas Ligand (Upstate, Cell signaling solutions, Lake Placid, NY, USA), anti-Fas antibody (R&D Systems, Inc. Minneapolis, MN, USA), all terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate 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, USA), antiguinea pig rhodamine-conjugated secondary antibody (Chemicon International, Temecula, CA, USA), and the materials indicated below.

Animals

Adult female Wistar rats (200–250 g) were kept in controlled conditions of light (12 h light–dark cycles) and temperature (20–25°C). Rats were fed standard lab chow and water ad libitum and kept in accordance with the NIH 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. Rats were monitored by daily vaginal smears over three consecutive cycles and killed in the morning of proestrus or diestrus. Groups of rats were ovariectomized (OVX) 2 weeks before the experiments under ketamine (75 mg/kg, i.p.) and xylacine (5 mg/kg, i.p.) anesthesia. Anterior pituitary glands were removed within minutes after decapitation and processed for cell culture.

Cell culture

A pool of anterior pituitary cells from 3 to 8 rats was used for each culture. Anterior pituitary glands were washed several times with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 μl/ml MEM amino acids, 2 mM glutamine, 5.6 μg/ml amphotericin B, 25 μg/ml gentamicin (DMEM-S), and 3 mg/ml bovine serum albumin (BSA). Then, the glands were cut into small fragments. Sliced fragments were dispersed enzymatically by successive incubations in DMEM-S–BSA containing 0.75% trypsin, 10% fetal bovine serum (FBS) previously treated with 0.025% dextran-0.25% charcoal (FBS–DCC) to remove steroids and 45 U/μl deoxyribonuclease type I. Finally, the cells were dispersed by extrusion through a Pasteur pipette in Krebs buffer without Ca^{2+} and Mg^{2+}. Dispersed cells were washed and resuspended in DMEM-S with 10% FBS–DCC. Cell viability as assessed by trypan blue exclusion was over 90%. Anterior pituitary cells were seeded onto coverslides in 24-well tissue culture plates (1×10^5 cells/ml/well) for the TUNEL assay or for flow cytometry analysis (FACS) (3×10^5 cells/ml/well). Anterior pituitary cells from intact rats were cultured for 24–48 h in DMEM-S with 10% FBS–DCC and then incubated for 24 h with an agonist anti-Fas antibody (Mab–Fas 1 μg/ml) [32] or recombinant Fas ligand (FasL 10 ng/ml) [33] in DMEM-S without serum.

In the case of OVX rats, the anterior pituitary cells were cultured for 24–48 h in DMEM-S with 10% FBS–DCC. After this period, the cells were incubated in the same medium containing vehicle (ethanol 1–10 μl/l), 17 β-estradiol (E2, 10^{-9} M, Sigma Chem. Co St Louis, MO, USA) and/or progesterone (P4, 10^{-6} M, Roussel-Uclaf Laboratory, Paris, France) for 48 h for FACS or incubated in DMEM-S without serum with the steroids for 24 h and then with Mab–Fas or FasL in the same media for a further 24 h for apoptosis determination.

Expression of Fas by flow cytometry

Cultured anterior pituitary cells from OVX rats were harvested with 0.025% trypsin–EDTA and washed in cold PBS. Cells were incubated in 0.1% PBS–BSA for 1 h at 37°C to stabilize cell membranes and then fixed with 0.1%

paraformaldehyde. Next, the cells were incubated with an anti-Fas FITC antibody (2 μg/10^5 cells, Santa Cruz Biotechnology, CA, USA) in PBS for 1 h at 37°C. For determination of total Fas (cell membrane plus cytoplasmatic expression), cells were permeabilized with 0.1% saponin (MP Biomedicals, Inc, OH, USA) for 10 min in the dark and at room temperature. Then, cells were incubated with an anti-Fas-fluorescein antibody (2 μg/10^5 cells) in PBS with 0.05% saponin for 1 h at 37°C. After the incubation with the antibody, the cells were washed, resuspended in PBS, and analyzed by FACS using a FACScan (Becton-Dickinson, NJ, USA). Data were analyzed using WinMDI 98 software. Cells were incubated with isotype control instead of primary antibody to determine the cut-off of Fas fluorescence.

Microscopic determination of DNA fragmentation by TUNEL

Briefly, after the culture period, cells were fixed with 4% paraformaldehyde in PBS for 10 min and permeabilized by microwave irradiation [34]. DNA strand breaks were labeled with digoxigenin-deoxyuridine triphosphate using terminal deoxynucleotidyl transferase (0.18 U/μl) according to the manufacturer's protocol. After an incubation in PBS with 10% normal donkey serum and 10% normal sheep serum for 40 min, the cells were incubated for 1 h with guinea pig rat prolactin (PRL) antiserum (1:2500) or guinea pig rat growth hormone (GH) antiserum (1:2000). Then, the slides were incubated with antidigoxigenin-fluorescein antibody (1:10) to detect incorporation of nucleotides into the 3′-OH end of damaged DNA and rhodamine-conjugated antiguinea pig secondary antibody (1:200). Slides were mounted with mounting medium for fluorescence (Vectashield, Vector Laboratories, Inc., Burlingame, CA, USA) containing 4′,6 diamidino-2-phenylindoledihydrochloride (DAPI) for DNA staining and visualized in a fluorescence microscope (Axiophot, Carl Zeiss Jena, Germany). The percentage of apoptotic lactotropes was calculated as [(TUNEL + PRL +)/total PRL +] × 100 and the percentage of apoptotic somatotropes as [(TUNEL + GH +)/total GH +] × 100.

Statistical analysis

The number of apoptotic cells identified by TUNEL was analyzed in slides from two to six independent experiments. Results were expressed as the percentage ± confidence limits (CL) of apoptotic cells of the total number of cells counted in each specific condition. 95% confidence intervals for proportions were analyzed by the χ^2 test. Data of the percentage of cells expressing Fas (by FACS) were expressed as means ± SE from three to five independent experiments and analyzed by two-way ANOVA followed by Tukey test. $P < 0.05$ was considered the cut-off point for significance.

Acknowledgments We would like to thank Mr. Pedro Cepero and Mr. Martín Brahamian for their kind help with animal care and handling.

Grant support This study was supported by grants from Agencia Nacional de Investigaciones Científicas y Tecnológicas, CONICET and Universidad de Buenos Aires, Argentina.

References

1. J. Song, E. Sapi, W. Brown, J. Nilsen, K. Tartaro, B.M. Kacinski, J. Craft, F. Naftolin, G. Mor, J. Clin. Invest. **106**, 1209–1220 (2000)
2. J. Nilsen, G. Mor, F. Naftolin, J. Neurobiol. **43**, 64–78 (2000)
3. J. Song, T. Rutherford, F. Naftolin, S. Brown, G. Mor, Mol. Hum. Reprod. **8**, 447–455 (2002)
4. E. Sapi, W.D. Brown, S. Aschkenazi, C. Lim, A. Munoz, B.M. Kacinski, T. Rutherford, G. Mor, J. Soc. Gynecol. Investig. **9**, 243–250 (2002)
5. K.A. Slot, M. Voorendt, M. de Boer-Brouwer, H.H. van Vugt, K.J. Teerds, J. Endocrinol. **188**, 179–192 (2006)
6. A. Strasser, P.J. Jost, S. Nagata, Immunity **30**, 180–192 (2009)
7. I. Sancho Martinez, A. Martin Villalba, Cell Cycle **8**, 838–842 (2009)
8. S. Nagata, P. Golstein, Science **267**, 1449–1456 (1995)
9. L. Xerri, E. Devilard, J. Hassoun, C. Mawas, F. Birg, Mol. Pathol. **50**, 87–91 (1997)
10. S.H. Lee, M.S. Shin, W.S. Park, S.Y. Kim, S.M. Dong, H.K. Lee, J.Y. Park, R.R. Oh, J.J. Jang, J.Y. Lee, N.J. Yoo, APMIS **107**, 1013–1019 (1999)
11. C. Lambert, A.M. Landau, J. Desbarats, Apoptosis **8**, 551–562 (2003)
12. N. Inoue, A. Maeda, F. Matsuda-Minehata, K. Fukuta, N. Manabe, J. Reprod. Dev. **52**, 723–730 (2006)
13. G. Jaita, M. Candolfi, V. Zaldivar, S. Zárate, L. Ferrari, D. Pisera, M.G. Castro, A. Seilicovich, Endocrinology **146**, 4737–4744 (2005)
14. M. Candolfi, V. Zaldivar, G. Jaita, A. Seilicovich, Front Horm. Res. **35**, 9–21 (2006)
15. Y. Oishi, M. Okuda, H. Takahashi, T. Fujii, S. Morii, Anat. Rec. **235**, 111–120 (1993)
16. A. Hashi, S. Mazawa, J. Kato, J. Arita, Endocrinology **136**, 4665–4671 (1995)
17. P. Yin, J. Arita, Neuroendocrinology **72**, 72–79 (2000)
18. V. Zaldivar, M.L. Magri, S. Zárate, G. Jaita, G. Eijo, D. Radl, J. Ferraris, D. Pisera, A. Seilicovich, Neuroendocrinology **90**, 292–300 (2009)
19. M.E. Freeman, In the neuroendocrine control of the ovarian cycle of the rat, in *The Physiology of Reproduction*, ed. by E. Knobil, J.D. Neill (Raven Press, New York, 1994)
20. P. Yin, J. Arita, Neuroendocrinology **76**, 272–282 (2002)
21. R.J. Rotello, R.C. Lieberman, R.B. Lepoff, L.E. Gerschenson, Am. J. Pathol. **140**, 449–456 (1992)
22. M. Candolfi, G. Jaita, V. Zaldivar, S. Zárate, L. Ferrari, D. Pisera, M.G. Castro, A. Seilicovich, Endocrinology **146**, 736–743 (2005)
23. L.A. Nolan, A. Levy, J. Neuroendocrinol. **21**, 457–464 (2009)
24. S.L. Asa, S. Ezzat, Endocr. Rev. **19**, 798–827 (1998)
25. T. Wells, Endocrinology **148**, 1943–1945 (2007)

26. S.R. Fox, R.E. Harlan, B.D. Shivers, D.W. Pfaff, Neuroendocrinology **51**, 276–283 (1990)
27. G.V. Childs, G. Unabia, P. Wu, Endocrinology **141**, 1560–1570 (2000)
28. T.D. Ladenfeld, J.M. Suttie, Endocrinology **125**, 1474–1478 (1989)
29. M. Degli Esposti, J. Tour, S. Ouasti, S. Ivanova, P. Matarrese, W. Malorni, R. Khosravi-Far, Mol. Biol. Cell **20**, 600–615 (2009)
30. V.N. Ivanov, Z. Ronai, T.K. Hei, J. Biol. Chem. **281**, 1840–1852 (2006)
31. G. Freiss, C. Puech, F. Vignon, Mol. Endocrinol. **12**, 568–579 (1998)
32. M.G. Cifone, R. De Maria, P. Roncaioli, M.R. Rippo, M. Azuma, L.L. Lanier, A. Santoni, R. Testi, J. Exp. Med. **180**, 1547–1552 (1994)
33. S. Jodo, S. Xiao, A. Hohlbaum, D. Strehlow, A. Marshak-Rothstein, S.T. Ju, J. Biol. Chem. **276**, 39938–39944 (2001)
34. S. Zárate, G. Jaita, V. Zaldivar, D.B. Radl, G. Eijo, J. Ferraris, D. Pisera, A. Seilicovich, Am. J. Physiol. Endocrinol. Metab. **296**, E664–E671 (2009)