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Apoptosis of Lactotrophs Induced by D2 Receptor Activation Is Estrogen Dependent

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Key Words
Anterior pituitary · Dopamine · Apoptosis · Estradiol · Lactotroph · D2 receptor

Abstract
Background/Aims: Dopamine (DA) inhibits prolactin release and reduces lactotroph proliferation by activating D2 receptors. DA and its metabolite, 6-hydroxydopamine (6-OHDA), induce apoptosis in different cell types. DA receptors and DA transporter (DAT) were implicated in this action. Considering that estradiol sensitizes anterior pituitary cells to proapoptotic stimuli, we investigated the effect of estradiol on the apoptotic action of DA and 6-OHDA in anterior pituitary cells, and the involvement of the D2 receptor and DAT in the proapoptotic effect of DA. ***Methods:*** Viability of cultured anterior pituitary cells from ovariectomized rats was determined by MTS assay. Apoptosis was evaluated by Annexin-V/flow cytometry and TUNEL. Lactotrophs were identified by immunocytochemistry. ***Results:*** DA induced apoptosis of lactotrophs in an estrogen-dependent manner. In contrast, estradiol was not required to trigger the apoptotic action of 6-OHDA. Cabergoline, a D2 receptor agonist, induced lactotroph apoptosis, while sulpiride, a D2 receptor antagonist, blocked DA-induced cell death. The blockade of DAT by GBR12909 did not affect the apoptotic action of DA, but inhibited 6-OHDA-induced apoptosis. ***Conclusion:*** These data show that DA, through D2 receptor activation, induces apoptosis of estrogen-sensitized anterior pituitary cells, and suggest that DA contributes to the control of lactotroph number not only by inhibiting proliferation but also by inducing apoptosis.
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Introduction

Dopamine (DA) is a catecholamine that participates in the control of many physiological processes, such as locomotion, cognition, motivation and neuroendocrine secretion [1]. The actions of DA are mediated by at least five different membrane receptors, which are grouped into two families: D1-like and D2-like. The D1-like family, which includes D1 and D5 receptors, is characterized by its coupling with Gs proteins leading to the activation of adenylyl cyclase. The D2-like family (D2, D3 and D4 receptors) interacts with Gi/Go proteins that reduce cAMP accumulation [2]. The DA transporter (DAT) controls the intensity and duration of dopaminergic neurotransmission by the reuptake of DA from the synaptic cleft [3].

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DA induces apoptosis in different cell types, such as neuroblastoma cells [4], striatal neurons [5] and thymocytes [6]. It has been shown that D1 or D2 receptor antagonists inhibit DA-induced cell death [7, 8], indicating that this action is exerted through activation of DA receptors. However, blockade of DAT inhibits DA-induced PC12 cell death, suggesting that the internalization of this catecholamine by DAT is needed to trigger apoptosis in this cell line [9]. Also, it has been suggested that DA auto-oxidation to 6-hydroxydopamine (6-OHDA), an oxidative metabolite that induces cell death in both neuronal and nonneuronal cell lines [10, 11], may mediate the cytotoxic action of DA [9].

In the anterior pituitary, DA tonically inhibits prolactin secretion. Hypothalamic neuroendocrine dopaminergic neurons release DA that reaches lactotrophs through the hypothalamic-pituitary portal vessels. After interacting with D2 receptors, this catecholamine induces membrane hyperpolarization and inactivation of voltage-gated calcium channels, reducing intracellular free calcium and inhibiting prolactin release. Also, D2 receptor activation inhibits prolactin gene expression and reduces lactotroph proliferation [1, 12]. Although DA induces apoptosis of GH3 cells, either the D2 receptor or DAT has been implicated in this action [13, 14]. This transporter was identified in anterior pituitary cells [15, 16].

Cell renewal in the anterior pituitary gland of female rats shows a characteristic rhythm along the estrous cycle. Cell proliferation peaks at estrus [17], whereas the maximum rate of apoptosis is observed at proestrus, the stage of the estrous cycle with the highest plasma levels of estrogens [18]. These observations suggest that the changes in circulating levels of gonadal steroids occurring over the estrous cycle are involved in anterior pituitary tissue homeostasis. In fact, in vitro studies from our laboratory showed that the apoptotic effects of TNF-α and FasL on anterior pituitary cells are estrogen-dependent and higher in cells from rats killed at proestrus than at diestrus [19, 20]. Also, in an in vivo model, the administration of estradiol to ovariectomized (OVX) rats induces apoptosis in the anterior pituitary gland and enables the proapoptotic action of endotoxemia [21].

Considering that estrogens sensitize anterior pituitary cells to different proapoptotic stimuli [19, 20, 21], we investigated the effect of estradiol on the apoptotic actions of DA in anterior pituitary cells, especially lactotrophs. Taking into account that the mechanism by which DA induces apoptosis of anterior pituitary cells has not been clearly defined, we also studied the involvement of the D2 receptor and DAT in DA-induced cell death. DA may induce apoptosis by its auto-oxidation to 6-OHDA [9], and therefore we explored the effect of this metabolite on anterior pituitary cell apoptosis.

Materials and Methods

Drugs

All drugs, media and supplements were obtained from Invitrogen (Carlsbad, Calif., USA), except Dulbecco's modified Eagle's medium (DMEM), EDTA, bovine serum albumin (BSA), 17β-estradiol, DA, 6-OHDA, GBR12909, normal donkey and sheep serum (Sigma, St. Louis, Mo., USA), fetal bovine serum (GBO, Buenos Aires, Argentina), all terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) reagents (Roche Molecular Biochemicals, Mannheim, Germany), [3-(4,5-dimethilthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) reagents (Promega, Madison, Wisc., USA), guinea pig rat prolactin antiserum (Dr. A. Parlow, National Hormone and Pituitary Program, Torrance, Calif., USA), anti-guinea pig rhodamine-conjugated secondary antibody (Chemicon International, Temecula, Calif., USA), fluorescein isothiocyanate (FITC) Annexin-V (BD Pharmingen, San Jose, Calif., USA), sulpiride (IVAX, Buenos Aires, Argentina) and cabergoline (kindly donated by Holliday-Scott, Argentina).

Animals

Adult female Wistar rats were kept in controlled conditions of light (12-hour 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. All the procedures were in compliance with the Ethical Committee of the School of Medicine, University of Buenos Aires. The rats were ovariectomized under ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) anesthesia 2 weeks before the experiments. Anterior pituitary glands were removed within minutes after decapitation.

Cell Culture

A pool of anterior pituitary cells from 5–8 OVX rats was used for each culture. Anterior pituitary glands were washed several times with 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 BSA. Then, the glands were cut into small fragments. Sliced fragments were dispersed enzymatically by successive incubations in DMEM-S with BSA, containing trypsin, deoxyribonuclease type I (45 U/μl) and 10% fetal calf serum, previously treated with 0.025% dextran-0.25% charcoal (FCS). The cells were then washed with Krebs-Ringer bicarbonate buffer free of Ca^{2+} and Mg^{2+} (KRBCMF), pH 7.4. Finally, the cells were dispersed in KRBCMF by extrusion through a Pasteur pipette. Dispersed cells were washed twice and resuspended in DMEM-S with 10% FCS. Cell dispersion yielded about 3–3.5 × 10^6 cells/gland. Cell viability as assessed by trypan blue exclusion was over 90%. The cells were seeded onto coverslides in 24-well tissue culture plates (1 × 10^5 cells/ml/well) for the TUNEL meth-

od or immunocytochemistry, onto 24-well tissue culture plates (3×10^5 cells/ml/well) for flow cytometric analysis (FACS) and onto 96-well tissue culture plates (5×10^4 cells/0.2 ml/well) for cell viability determination. Cells were cultured for 72 h in DMEM-S with 10% FCS. Then, the cells were cultured for 24 h in fresh medium containing vehicle (VEH, ethanol 1 μl/l) or 17β-estradiol (E2, 10^{-9} M), and for a further 24 h in the same media without serum. After this period, the cells were incubated in the same media containing different drugs according to each experiment.

Determination of Cell Viability

Metabolic activity of cultured cells was determined by the MTS assay. 20 μl of reaction solution containing MTS (final concentration 333 μg/ml) and an electron-coupling reagent (phenazine ethosulfate, final concentration 25 μM) were added to each well containing 100 μl of culture medium. After 3 h at 37°C, the OD was read in a microplate spectrophotometer at a wavelength of 495 nm.

Detection of Apoptosis by FITC Annexin-V Staining

Cells were harvested with 0.05% trypsin and washed twice with cold PBS. Then, they were suspended in binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) at a concentration of 3×10^5 cells/0.1ml. Then, the cells were incubated with 5 μl FITC Annexin-V and 10 μl propidium iodide (PI, 50 μg/ml) for 15 min in darkness. Cells were immediately analyzed by flow cytometry (FACS, Becton-Dickinson FACScalibur). Annexin-V-positive/PI-negative cells were considered early apoptotic cells, 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.

Microscopic Determination of DNA Fragmentation by TUNEL and Immunocytochemistry

After the culture period, cells were fixed with 4% paraformaldehyde for 10 min and permeabilized by microwave irradiation. DNA strand breaks were labeled with digoxigenin-deoxyuridine triphosphate using terminal deoxynucleotidyl transferase (0.18 U/μl) according to the manufacturer's protocol. After incubation with 10% normal donkey serum and 10% normal sheep serum in PBS for 90 min, cells were incubated for 1 h with guinea pig rat prolactin antiserum (1:1,500). Then, 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 anti-guinea pig secondary antibody (1:200) in the same buffer. Control slides were incubated with normal guinea pig serum instead of primary antibody. Slides were mounted with Vectashield (Vector Laboratories, Inc., Burlingame, Calif., USA) containing 4′,6 diamidino-2-phenilindoledihydrocloride for DNA staining and visualized in a fluorescent light microscope (Axiophot; Carl Zeiss Jena, Germany). The percentage of lactotrophs determined by immunocytochemistry represented 25–40% of the population of anterior pituitary cells. The percentage of apoptotic anterior pituitary cells was calculated as (TUNEL-positive cells/total anterior pituitary cells) ×100; and the percentage of apoptotic lactotrophs was calculated as (TUNEL-positive prolactin-immunoreactive cells/prolactin-immunoreactive cells) ×100 [19].

Statistical Analysis

Each experiment was performed at least twice. Viability data were expressed as mean ± SE and evaluated by one-way ANOVA followed by Dunnett's test. The percentage of apoptotic cells determined by FACS was expressed as mean ± SE and evaluated by two-way ANOVA followed by Tukey's test. Differences were considered significant if $p < 0.05$.

The number of apoptotic cells identified by TUNEL was determined in duplicate slides from independent experiments. Results were expressed as the percentage ± 95% confidence interval (CI) of apoptotic cells of the total number of cells counted in each specific condition. Differences between proportions were analyzed by χ^2.

Results

Effect of DA on the Apoptosis of Anterior Pituitary Cells

We evaluated the action of increasing concentrations of DA on the viability of anterior pituitary cells from OVX rats cultured with or without E2. The viability of anterior pituitary cells from OVX rats was not modified by DA (0.1–10 μM). However, in the presence of E2, DA at concentrations of 1 μM or higher decreased cell viability (data not shown). Hence, in the subsequent experiments DA at a concentration of 1 μM was used.

To test whether the reduction of anterior pituitary cell viability induced by DA in the presence of E2 is due to apoptosis, we evaluated the percentage of cells stained with Annexin-V by FACS. E2 per se did not significantly modify the percentage of apoptotic cells. DA increased the percentage of Annexin-V-positive cells only when the cells were cultured with E2 (fig. 1). Also, we determined the DA proapoptotic effect on lactotrophs by TUNEL and immunocytochemistry to identify apoptotic prolactin-immunoreactive cells. DA increased the percentage of TUNEL-positive cells and TUNEL-positive lactotrophs only when they were cultured in the presence of E2 (fig. 2). DA did not modify the percentage of TUNEL-positive nonprolactin-immunoreactive cells from OVX rats (data not shown), but it significantly increased the percentage of apoptotic nonprolactin-immunoreactive cells incubated in the presence of E2 (E2: 0.8% vs. E2+DA: 7.7%; $p < 0.01$, χ^2).

Effect of 6-OHDA on the Apoptosis of Anterior Pituitary Cells

Considering that DA can be oxidized to 6-OHDA, we evaluated the effect of this metabolite on the viability of anterior pituitary cells cultured in the presence or absence of E2. 6-OHDA at concentrations of 1 μM or high-

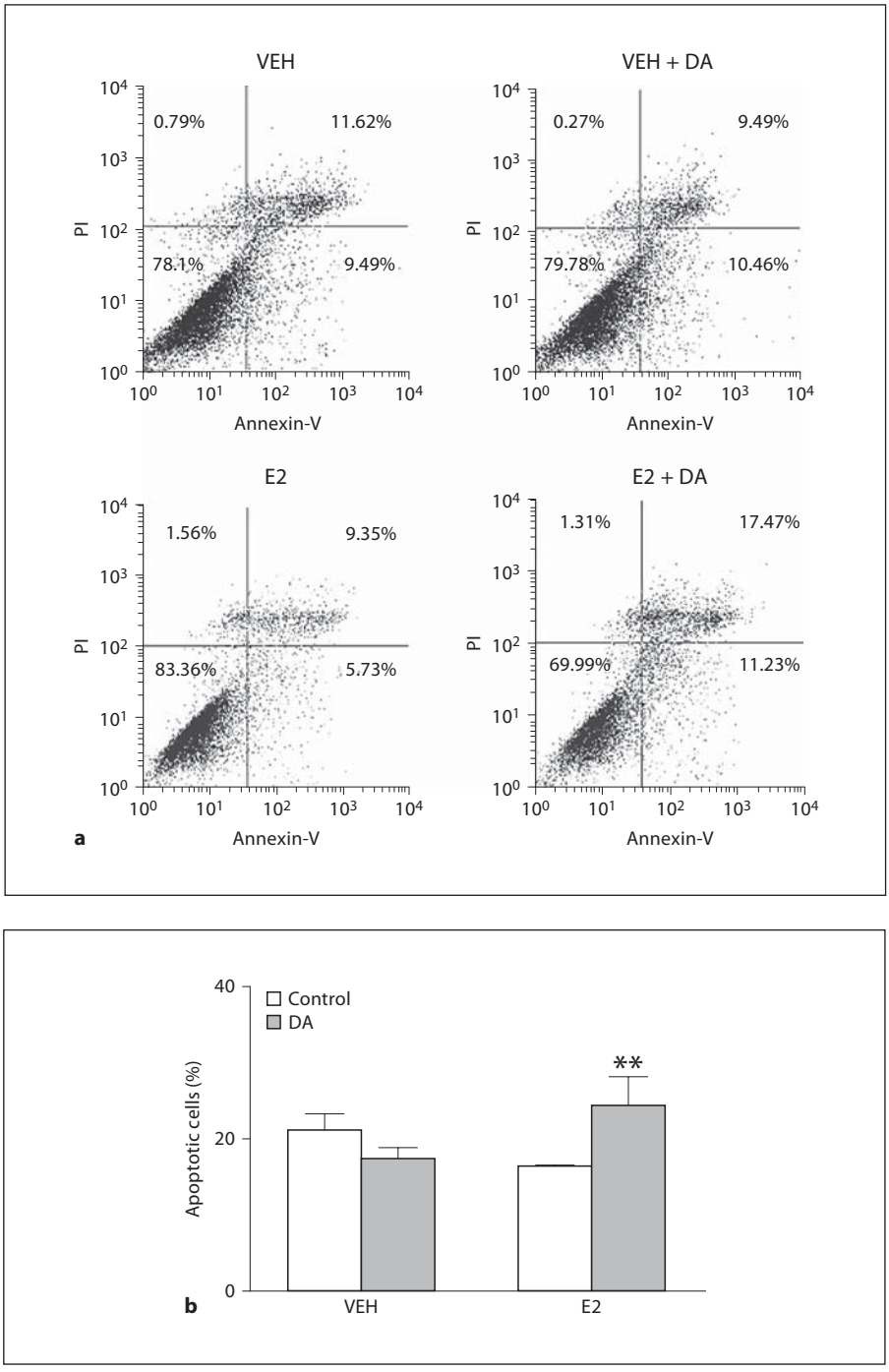

Fig. 1. Effect of DA on anterior pituitary cell apoptosis. Anterior pituitary cells from OVX rats cultured with VEH (ethanol, 1 μl/l) or E2 (10^{-9} M) for 48 h were incubated with DA (10^{-6} M) for 4 h. The percentage of apoptosis was determined by flow cytometry. **a** Contour diagram represents apoptotic cells marked with Annexin-V and PI. The lower left quadrant of each panel shows the viable cells, Annexin-V negative/PI negative. The upper right quadrant of each panel contains the nonviable, late-stage apoptotic cells, Annexin-V positive/PI positive. The lower right quadrant of each panel represents the early apoptotic cells, Annexin-V positive/PI negative. The upper left quadrant of each panel represents the necrotic cells, Annexin-V negative/PI positive. In each panel, the sum of the upper right quadrant plus the lower right quadrant was considered total apoptosis. **b** Each column represents the mean ± SE of the percentage of apoptotic cells (n = 4 wells) from a representative experiment. Data were analyzed by two-way ANOVA, followed by Tukey's test. ** $p < 0.01$ vs. respective control without DA.

er decreased the metabolic activity of anterior pituitary cells cultured either with or without E2 after 4 h of incubation (data not shown). Also, 6-OHDA increased the percentage of TUNEL-positive anterior pituitary cells (fig. 3a) and TUNEL-positive lactotrophs (fig. 3b). However, the presence of E2 decreased the apoptotic effect of 6-OHDA (fig. 3).

Involvement of the D2 Receptor in the Proapoptotic Effect of DA

To test whether DA induces apoptosis of anterior pituitary cells through its interaction with D2 receptors, the apoptotic effect of DA was evaluated in anterior pituitary cells cultured with E2 and incubated in the presence of sulpiride, a specific D2 receptor antagonist. Sulpiride (0.1

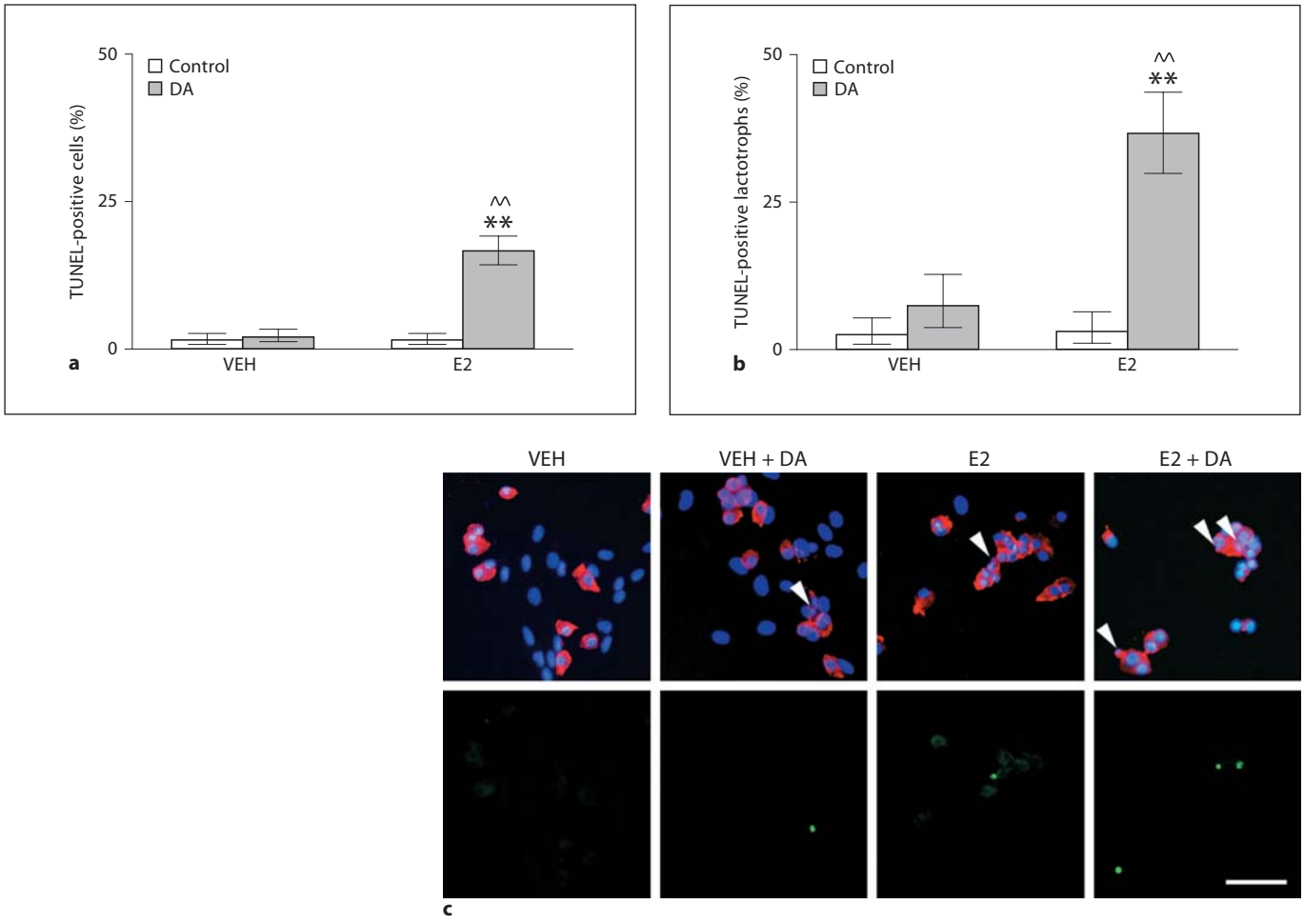

Fig. 2. Effect of DA on the apoptosis of anterior pituitary cells and lactotrophs. Anterior pituitary cells cultured with VEH (ethanol, 1 μl/l) or E2 (10^{-9} M) for 48 h were incubated with DA (10^{-6} M) for 4 h. Each column represents the percentage of TUNEL-positive cells ± CI (≥2,000 cells/group; **a**), or the percentage of TUNEL-positive lactotrophs ± CI (≥500 cells/group; **b**). Data from at least two separate experiments were analyzed by χ^2. ** $p < 0.01$ vs. respective control without DA; ^^ $p < 0.01$ vs. respective control without E2. **c** Representative anterior pituitary cells showing immunoreactivity for prolactin (red) counterstained with 4′,6 diamidino-2-phenilindoledihydrocloride (blue, upper panels) and DNA fragmentation by TUNEL (lower panels). Arrowheads indicate apoptotic cells. Scale bar: 50 μm.

μM) per se did not modify the percentage of TUNEL-positive cells and TUNEL-positive lactotrophs, but blocked the apoptosis induced by DA in total anterior pituitary cells and lactotrophs cultured in the presence of E2 (fig. 4). Also, cabergoline (1 μM), a specific D2 receptor agonist, increased the percentage of TUNEL-positive cells (fig. 5a) and TUNEL-positive lactotrophs (fig. 5b) only when the cells were cultured in the presence of E2.

Involvement of DAT in the Proapoptotic Effect of DA and 6-OHDA

To evaluate whether DAT is involved in the apoptotic action of DA and 6-OHDA on anterior pituitary cells, we investigated their effects in the presence of GBR12909, a DAT-specific inhibitor. GBR12909 (10 nM) per se did not modify the percentage of TUNEL-positive cells and TUNEL-positive lactotrophs, and did not affect the apoptotic action of DA (fig. 6a, b). However, GBR12909 inhibited the 6-OHDA-induced apoptosis (fig. 6c, d).

Discussion

The anterior pituitary gland undergoes constant cell renewal by processes of mitosis, apoptosis and cell differentiation [22]. During the estrous cycle, the rate of apo-

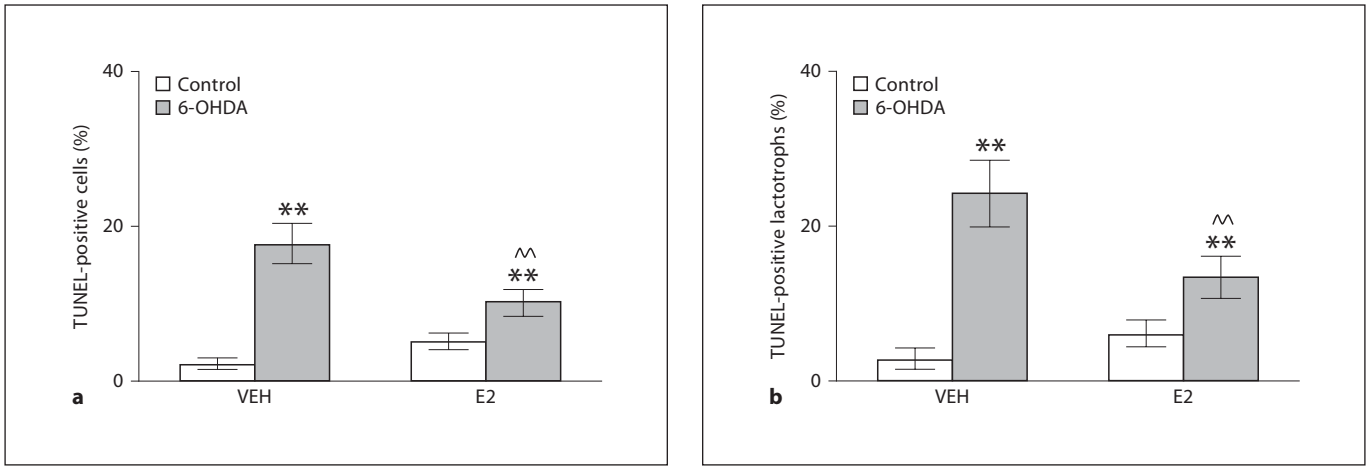

Fig. 3. Effect of 6-OHDA on the apoptosis of anterior pituitary cells and lactotrophs. Cells cultured with VEH (ethanol, 1 μl/l) or E2 (10^{-9} M) for 48 h were incubated with 6-OHDA (10^{-6} M) for 4 h. Each column represents the percentage of TUNEL-positive cells ± CI (≥2,000 cells/group; **a**), or the percentage of TUNEL-positive lactotrophs ± CI (≥500 cells/group; **b**). Data from at least two separate experiments were analyzed by χ^2. ** $p < 0.01$ vs. respective control without 6-OHDA; ^^ $p < 0.01$ vs. respective control without E2.

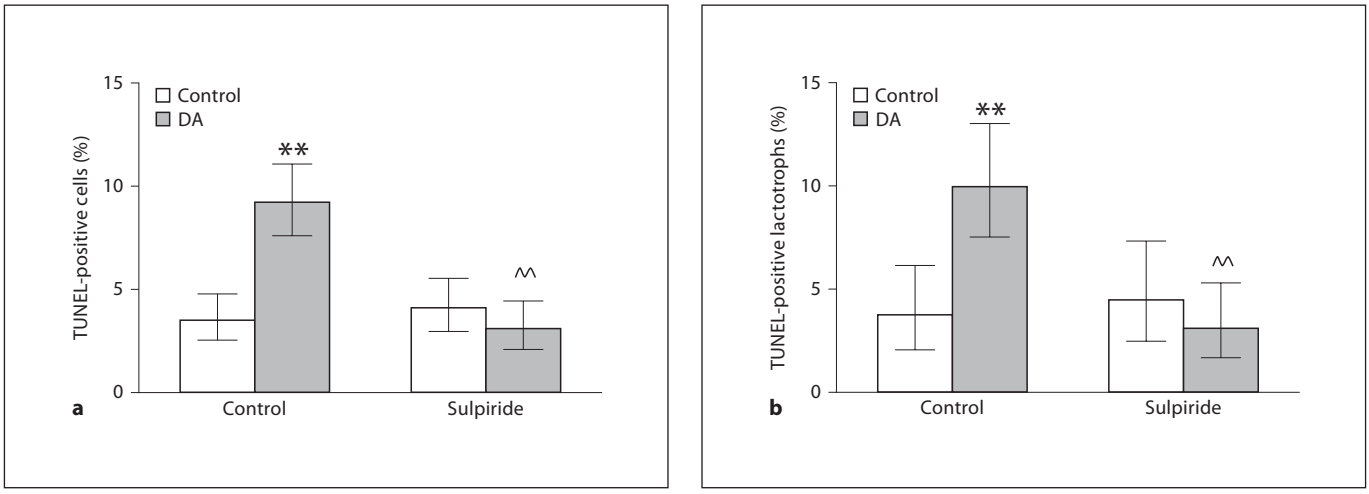

Fig. 4. Effect of sulpiride on the apoptosis induced by DA. Anterior pituitary cells from OVX rats were cultured with E2 (10^{-9} M) for 48 h. Cells were preincubated with or without sulpiride (10^{-7} M) for 30 min and then incubated with or without DA (10^{-6} M) in the presence or absence of sulpiride for 4 h. Each column represents the percentage of TUNEL-positive cells ± CI (≥2,000 cells/group; **a**), or the percentage of TUNEL-positive lactotrophs ± CI (≥500 cells/group; **b**). Data from at least two separate experiments were analyzed by χ^2. ** $p < 0.01$ vs. respective control without DA; ^^ $p < 0.01$ vs. respective control without sulpiride.

ptosis correlates with circulating levels of estrogens [18]. In the present study, we show that DA induces apoptosis of anterior pituitary cells and lactotrophs only in the presence of E2, suggesting that estrogens exert a permissive action on the apoptosis induced by DA in these cells. In fact, we previously observed that estrogens increase the sensitivity of anterior pituitary cells to different apoptotic stimuli [19–21]. We also showed that lipopolysaccharide-induced apoptosis in the anterior pituitary gland is higher at proestrus than at other stages of the estrous

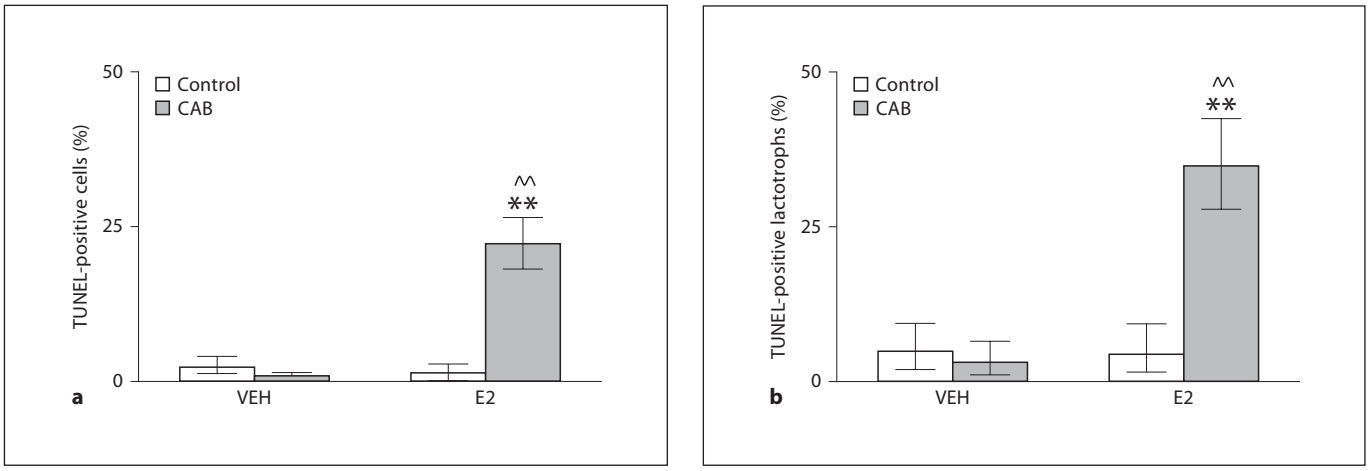

Fig. 5. Effect of cabergoline on the apoptosis of anterior pituitary cells and lactotrophs. The cells were cultured with VEH (ethanol, 1 μl/l) or E2 (10^{-9} M) for 48 h. Then, they were incubated with cabergoline (CAB, 10^{-6} M) for 4 h. Each column represents the percentage of TUNEL-positive cells ± CI (≥2,000 cells/group; **a**), or the percentage of TUNEL-positive lactotrophs ± CI (≥500 cells/group; **b**). Data from at least two separate experiments were analyzed by χ^2. ** $p < 0.01$ vs. respective control without CAB; ^^ $p < 0.01$ vs. respective control without E2.

cycle [21]. Endogenous variations in circulating levels of estrogens may modulate apoptosis in the anterior pituitary gland by modifying its responsiveness to proapoptotic signals. DA may be one of these signals contributing to the apoptosis of lactotrophs previously sensitized by high levels of estrogens. In this regard, the secretory response of lactotrophs to DA has been observed to be modulated by gonadal steroids and to depend on the stage of the estrous cycle [23, 24]. Although estrogens are associated with proliferative actions on lactotrophs, E2 can also exert antiproliferative effects. Indeed, it has been reported that incubation of anterior pituitary cells with E2 for up to 72 h reduces the mitogenic action of insulin and IGF-1 on lactotrophs [25, 26]. Other findings suggest that the proliferative effect of estrogens on lactotrophs could be mediated by paracrine factors [27]. Since E2 stimulates the local release of cytokines [22], it is possible to speculate that paracrine mediators could be involved in the sensitizing action of E2 to proapoptotic stimuli. About 2.5% of lactotrophs proliferate at estrus [17] and, therefore, a similar number of these cells might die to maintain the size of the lactotroph subpopulation. Even though the percentage of anterior pituitary cells renewed in each estrous cycle is low, dysregulation of this process may have consequences on tissue homeostasis in cycling females.

The main therapeutic option for the treatment of prolactinomas is the administration of D2 receptor agonists, such as bromocriptine or cabergoline, which inhibit prolactin secretion and reduce the size of the tumors. However, a subset of patients with prolactinomas does not respond to pharmacological therapy with dopaminergic agonists. DA agonist resistant prolactinomas have been associated not only with lack of D2 receptor, but also with alterations in signaling pathways downstream of the D2 receptor activation [28]. Our results indicate that DA-induced apoptosis of anterior pituitary cells requires the presence of E2. The sensitizing action of estrogens may involve the modulation of D2 receptor expression [29, 30] and/or changes in the signal transduction pathways triggered by D2 receptor activation. The permissive effect of E2 on DA-induced apoptosis could be exerted not only on lactotrophs but also on other anterior pituitary cell types where DA receptors are expressed, such as corticotrophs [31], somatotrophs [32], and gonadotrophs [33].

DA has been reported to induce apoptosis of anterior pituitary cells [13–15], but the mechanism that triggers this process remains controversial. Jaubert et al. [14, 15] reported that in anterior pituitary cells from postlactating rats and GH3 cells, a somatolactotroph cell line lacking D2 receptors, DA-induced apoptosis is mediated by DAT. However, An et al. [13] showed that DA induced apoptosis of GH3 cells only when they were transfected with D2 receptors. In the present study, we observed that cabergoline, a D2 receptor agonist, mimicked the effect of DA, inducing apoptosis of anterior pituitary cells and lactotrophs in an estrogen-dependent manner. Also, sul-

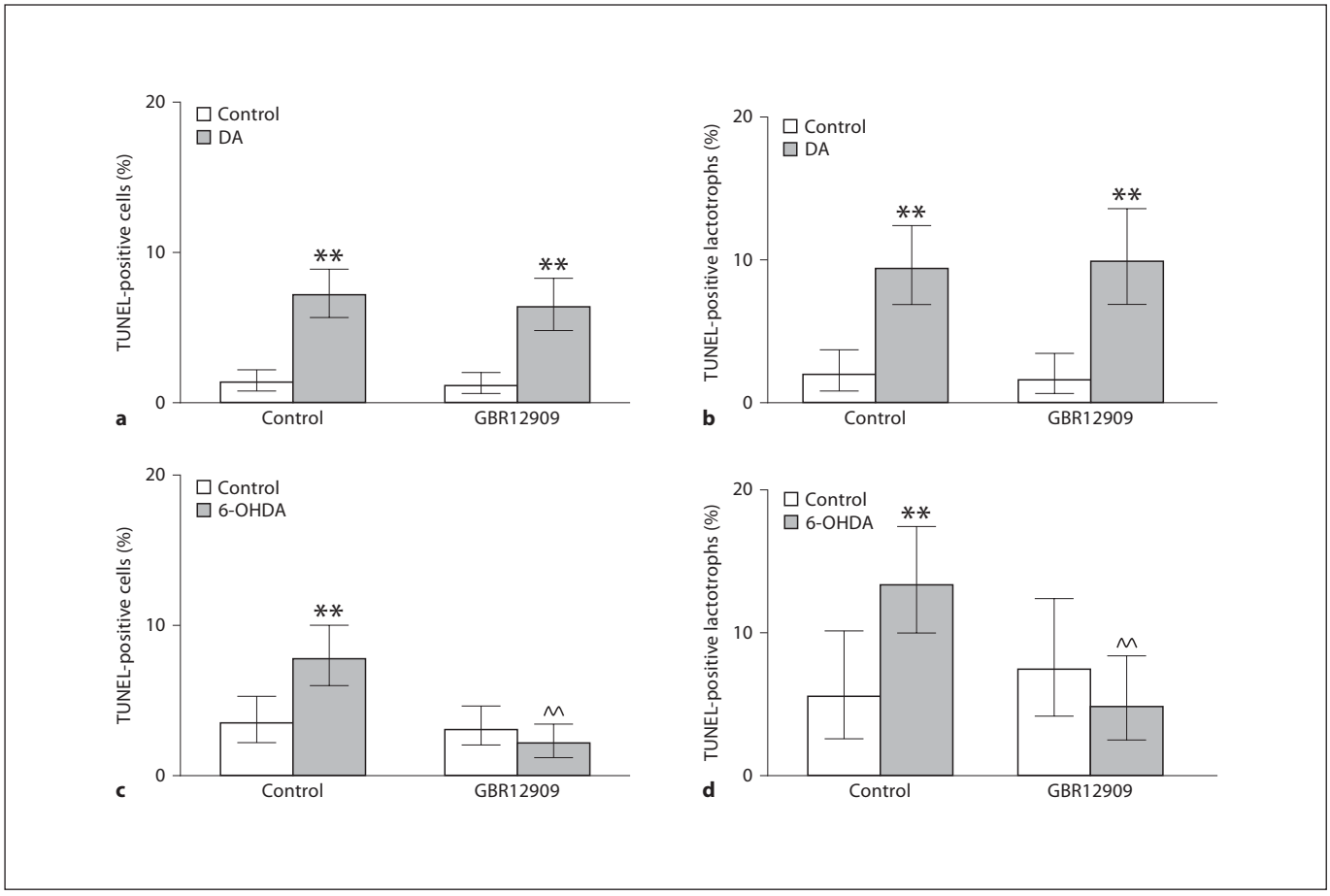

Fig. 6. Effect of GBR12909 on the apoptosis of anterior pituitary cells and lactotrophs induced by DA or 6-OHDA. **a**, **b** Anterior pituitary cells cultured in the presence of E2 (10^{-9} M) were preincubated with or without GBR12909 (10^{-8} M) for 30 min. Then, the cells were incubated with or without DA (10^{-6} M) in the presence or absence of GBR12909 for 4 h. **c**, **d** Anterior pituitary cells cultured without E2 were preincubated with or without GBR12909 (10^{-8} M) for 30 min. Then, the cells were incubated with or without 6-OHDA (10^{-6} M) in the presence or absence of GBR12909 for 4 h. Each column represents the percentage of TUNEL-positive cells ± CI (≥2,000 cells/group; **a**, **c**), or the percentage of TUNEL-positive lactotrophs ± CI (≥500 cells/group; **b**, **d**). Data from at least two separate experiments were analyzed by χ^2. ** $p < 0.01$ vs. respective control without DA or 6-OHDA; ^^ $p < 0.01$ vs. respective control without GBR12909.

piride, a specific D2 receptor antagonist, blocked the apoptotic action of DA. These results strongly suggest that DA induces apoptosis of anterior pituitary cells, especially lactotrophs, by activating the D2 receptor. In contrast to the findings reported by Jaubert et al. [14, 15], in our experimental conditions, the blockade of DAT failed to affect the apoptotic action of DA. Although the participation of DAT in anterior pituitary cell death under specific conditions cannot be excluded, DAT-deficient mice show anterior pituitary hypoplasia and reduction in the number of lactotrophs [34], suggesting that DA would be interacting with D2 receptors to reduce lactotroph proliferation and/or increase lactotroph apoptosis. Furthermore, D2 receptor-deficient mice develop pituitary hyperplasia and an increased number of lactotrophs [35, 36], indicating the involvement of this receptor in the control of lactotroph population.

The effect of DA on other anterior pituitary cell types that potentially may mediate its proapoptotic action cannot be ruled out. However, this catecholamine induces apoptosis of somatolactotroph GH3 cells transfected with D2 receptors [13], suggesting that the apoptotic action of DA may be directly exerted on lactotrophs. The inhibition of lactotroph proliferation induced by D2 receptor agonists has been reported to be mediated by TGF-β1 release from lactotrophs [12]. Since this cytokine induces

apoptosis of anterior pituitary cells [37], we cannot rule out the involvement of TGF-β1, acting as an autocrine factor in DA-induced apoptosis.

Evidence points to 6-OHDA as an endogenous neurotoxin. Dopaminergic neurons contain significant levels of DA, hydrogen peroxide and free iron that can lead to 6-OHDA formation through a nonenzymatic reaction between these elements [9]. Some studies suggest that 6-OHDA needs to be internalized by DAT to produce its cytotoxic effect [38, 39]. However, extracellular auto-oxidation of 6-OHDA was reported to induce oxidative stress and apoptosis [10]. Our results show that the blockade of DAT by GBR12909 inhibits the proapoptotic action of 6-OHDA on anterior pituitary cells, suggesting that its internalization by DAT is required to trigger this effect. This finding and the estrogen independence of 6-OHDA-induced apoptosis of lactotrophs indicate that, in our experimental conditions, the apoptotic effect of DA is not mediated by its auto-oxidation to 6-OHDA. The lower apoptotic effect of 6-OHDA observed when the anterior pituitary cells were incubated in the presence of E2 could be due to inhibition of DAT activity induced by estrogens, as it has been reported [40, 41].

In conclusion, this study suggests that the control of the number of lactotrophs by D2 receptor activation involves not only the inhibition of lactotroph proliferation [1, 12], but also the induction of apoptosis. The presence of E2 seems to be required to trigger the apoptotic action of DA in lactotrophs.

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