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Estradiol Promotes Luteal Regression Through a Direct Effect on the Ovary and an Indirect Effect From the Celiac Ganglion via the Superior Ovarian Nerve

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Abstract

There is evidence suggesting that estradiol (E_2) regulates the physiology of the ovary and the sympathetic neurons associated with the reproductive function. The objective of this study was to investigate the effect of E_2 on the function of late pregnant rat ovaries, acting either directly on the ovarian tissue or indirectly via the superior ovarian nerve (SON) from the celiac ganglion (CG). We used in vitro ovary (OV) or ex vivo CG-SON-OV incubation systems from day 21 pregnant rats. Various concentrations of E_2 were added to the incubation media of either the OV alone or the ganglion compartment of the CG-SON-OV system. In both experimental schemes, we measured the concentration of progesterone in the OV incubation media by radioimmunoassay at different times. Luteal messenger RNA (mRNA) expression of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 20α-hydroxysteroid dehydrogenase (20α-HSD) enzymes, respectively, involved in progesterone synthesis and catabolism, and of antiapoptotic B-cell lymphoma 2 (Bcl-2) and proapoptotic Bcl-2-associated X protein (Bax), were measured by reverse transcriptase–polymerase chain reaction (RT-PCR) at the end of the incubation period. Estradiol added directly to the OV incubation or to the CG of the CG-SON-OV system caused a decline in the concentration of progesterone accumulated in the incubation media. In addition, E_2, when added to the OV incubation, decreased the expression of 3β-HSD and the ratio of Bcl-2/Bax. We conclude that through a direct effect on the OV, E_2 favors luteal regression at the end of pregnancy in rats, in association with neural modulation from the CG via the SON.

Keywords

estradiol, pregnancy, corpus luteum, peripheral nervous system, celiac ganglion.

Introduction

There is evidence that demonstrates a functional interaction between the endocrine and peripheral nervous systems on the physiology of the ovary during pregnancy.[1-5] The ovary is innervated by the ovarian plexus and the superior ovarian nerve (SON), which is most relevant impacting ovarian steroidogenesis. The SON is mainly constituted by adrenergic fibers, most of which originate in the celiac ganglion (CG).[6] This ganglion is included in the sympathetic prevertebral chain; it contains principal neurons, interneurons, and has a profuse capillary plexus that forms a microcirculation among the different ganglionic structures.[7–9] The sympathetic ganglia possess a variety of receptors and neurotransmitters, including catecholamines[6] and neuropeptides.[10] These ganglionic neurons are active points of concurrency for hormone signals.[11]

At the end of pregnancy in the rat, it is necessary that the corpora lutea, which are the dominant steroidogenic tissues within the ovaries, undergo functional regression that is highlighted by the decline in the capacity to produce progesterone in order for parturition to ensure.[12] The structural involution of the corpora lutea is, however, clearly evident only after parturition and it is manifested with reduction in size and weight as a consequence

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Table 1. Primer Sequences and Reaction Conditions Used in the PCR Amplification of the Various cDNAs

Gene	Sense of Primers (5′-3′)	Size of Amplification Product (pb)	PCR Cycles
3β-HSD	GTCTTCAGACCAGAAACCAAG TAAGGCACAAGTATGCAG	447	35
20α-HSD	TTCGAGCAGAACTCATGGCTA CAACCAGGTAGAATGCCATCT	440	35
Bcl-2	AGAGGGGCTACGAGTGGGAT CTCAGTCATCCACAGGGCGA	454	35
Bax	GATTGCTGACGTGGACACGGACT TCAGCCCATCTTCTTCCA	473	40
S16	TCCAAGGGTCCGCTGCAGTC CGTTCACCTTGATGAGCCCATT	100	35

Abbreviations: cDNA, complementary DNA; PCR, polymerase chain reaction; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 20α-HSD, 20α-hydroxysteroid dehydrogenase.

[a] All reactions were carried out with the following cyclic parameters for 3β-HSD, 20α-HSD, B-cell lymphoma 2 (Bcl-2), and S16: 95°C for 1 minute, 59°C 1 minute, and 72°C 1 minute and for Bcl-2-associated X protein (Bax): 95°C for 1 minute, 56°C 1 minute, and 72°C 1 minute. All the reactions were terminated with a 5-minute extension at 72°C.

of an apoptotic phenomenon.[12] There are several apoptotic-associated genes linked to luteal regression that are upregulated before parturition likely as a consequence of a decline in serum progesterone levels.[13] The molecular mechanisms that trigger luteal demise are however not yet entirely understood.

Previous studies have suggested that in the rat, estradiol (E_2) is a key hormone regulating reproductive processes when acting on the corpus luteum and on the sympathetic neurons associated with the reproductive function. During mid-pregnancy, E_2 is required for luteal survival.[14,15] At the end of pregnancy, E_2 levels increase in the ovarian vein and in the general circulation, when compared with the values maintained throughout pregnancy.[16] It has been proposed that the increase in the serum concentration of E_2 at the end of gestation is responsible for increased synthesis of prostaglandin F2α and luteal expression of prostaglandin F2α receptors in association with functional regression of the corpora lutea.[17]

Circulating E_2 may gain access to the CG impacting indirectly the physiology of the ovary because of the known connection between the CG and the ovary via the SON.[18] In fact, the CG is exposed to systemic bioactive substances as it has very permeable capillaries.[9] Because the specific luteal function of E_2 in rats at the end of pregnancy is not entirely known, we utilized an in vitro ovary (OV) and ex vivo CG-SON-OV incubation systems obtained from day 21 pregnant rats, added E_2 in the OV compartment or in the CG compartment, and evaluated the progesterone biosynthetic capacity of the ovaries in an attempt to define direct versus neural-mediated effects of E_2 on the corpora lutea of the late pregnant rats.

Materials and Methods

Animals

Virgin Holtzman strain female rats weighing 250 g were used in all the experiments. Animals had free access to food (Cargill SACI, Saladillo, Buenos Aires, Argentina) and water. They were kept in a light- (lights on from 0700 to 1900 hours) and temperature-controlled room (24°C ± 2°C). To induce pregnancy, female rats were caged individually with fertile males on the afternoon of proestrus. Positive mating was verified on the following morning by identifying sperm or copulation plugs in the vagina. This day was designated as day 0 of pregnancy. In our laboratory, rats usually give birth on day 22. Groups of 6 animals on day 21 of pregnancy were used for each experimental procedure. Each experiment included an experimental and a control group. Animals were handled according to the procedures approved in the UFAW Handbook on the Care and Management of Laboratory Animals. The experimental protocol was approved by the National University of San Luis Animal Care and Use Committee (resolution N° 342/10; B-30/08).

Reagents

The chemicals and other reagents of analytical grade used for this study were from Sigma Chemical Co (St. Louis, Missouri). 1,2,6,7-[3H]-Progesterone (107.0 Ci/mmol) was provided by New England Nuclear Products (Boston, Massachusetts).

Experimental Procedure

In the first experiment, we examined the effects of E_2 on luteal regression when added in vitro on the OV incubation media; whole ovaries were removed from the rats and incubated in Krebs Ringer bicarbonate buffer, pH 7.4 with the addition of 0.1 mg glucose/mL and 0.1 mg albumin/mL at 37°C in an atmosphere composed of 95% of O_2 and 5% of CO_2. To the experimental groups 10^{-6}, 10^{-8}, or 10^{-9} mol/L E_2 was added to the ovarian incubation medium.

In the second experiment, we analyzed the effects of E_2 on luteal regression acting indirectly via neural, the ex vivo CG-SON-OV system was used. The surgical procedure to remove the organ system was carried out according to Casais and colleagues.[1] The CG-SON-OV system was removed and placed in a cuvette with 2 separate compartments, one for the ovary and the other for the ganglion, connected by the SON, maintaining the nerve wet with the working solution. The system was stabilized by incubation in a metabolic bath at 37°C for 30 minutes in an atmosphere composed by 95% O_2 and 5% CO_2. The end of the preincubation period was considered as incubation time 0. At this time, the buffer was changed in both compartments and ascorbic acid (0.1 mg/mL in Krebs Ringer) was added as an antioxidant agent to the ganglion compartment. The experimental groups included the CG-SON-OV system with the addition of 10^{-6}, 10^{-8}, or 10^{-9} mol/L E_2 to the ganglion compartment.

Periodic extractions of the ovary incubation liquid (250 μL) from both experimental schemes were carried out at 30, 60, 120, 180 and 240 minutes and were kept at −20°C until progesterone was determined by radioimmunoassay (RIA). The corresponding corrections were made in all cases, taking into consideration the volume extracted in each tested period. At the end of the total incubation period, the corpora lutea of the control group and of the groups receiving E_2 were isolated on ice

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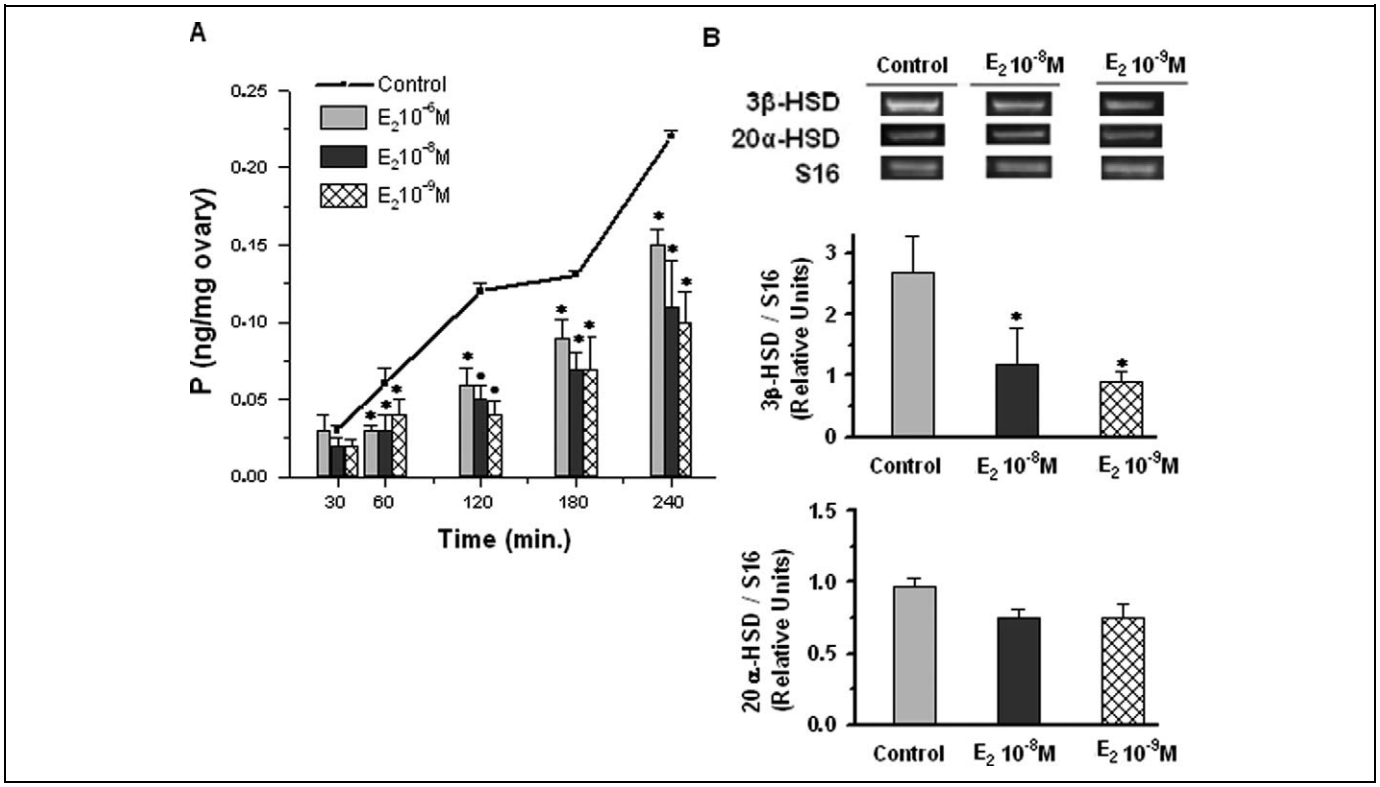

Figure 1. A, Direct effect of estradiol (E_2) on ovarian progesterone (P) released, expressed as ng/mg ovary, in 21-day pregnant rats. Results are expressed as mean ± SEM of 6 animals per group. One-way analysis of variance followed by Tukey's test was used. *$P < .05$ • $P < .005$). B, Direct effect of E_2 on luteal mRNA expression of enzymes 3β-HSD and 20α-HSD in 21-day pregnant rats. The gel photographs were quantified using ImageJ software and expressed as arbitrary units. S16 was used as the housekeeping gene. Results are expressed as mean ± SEM (n = 3). One-way analysis of variance followed by Tukey test was used. *$P < .05$. 3β-HSD indicates 3β-hydroxysteroid dehydrogenase; 20α-HSD, 20α-hydroxysteroid dehydrogenase; SEM, standard error of the mean; mRNA, messenger RNA.

under stereoscopic lens and frozen at −80°C for RNA isolation and determination of the messenger RNA (mRNA) expression levels of 3β-hydroxysteroid dehydrogenase (3β-HSD), 20α-hydroxysteroid dehydrogenase (20α-HSD), B-cell lymphoma 2 (Bcl-2), and Bcl-2-associated X protein (Bax).

Progesterone Assay

Progesterone was measured by RIA. The sensitivity of the assay was less than 5 ng progesterone/mL of serum, and the inter- and intra-assay coefficients of variation were less than 10%. This assay has been previously validated.[19] The results were expressed as nanograms of progesterone per milligram of ovarian tissue (ng/mg ovary).

RNA Isolation and Reverse Transcriptase–Polymerase Chain Reaction Analysis

Total luteal RNA was isolated using TRIZOL Reagent (Invitrogen Life Technologies, Rockville, Maryland), according to the manufacturer's instructions. Two micrograms of total RNA were reverse transcribed at 37°C using random primers and M-MLV Reverse Transcriptase ([RT] Promega, Madison, Wisconsin) in a 26-μL reaction mixture. Fragments coding for S16, 3β-HSD, 20α-HSD, Bcl-2, and Bax were amplified by PCR in a 50-μL reaction solution containing 0.2 mmol/L deoxynucleotide triphosphates (dNTPs), 1.5 mmol/L $MgCl_2$, 1.25 U of Taq polymerase, 50 pmol of each rat-specific oligonucleotide primer and RT-generated complementary DNA ([cDNA] 1/5 of RT reaction). The sequences of the specific primers are shown on Table 1. The amplification of the cDNA was performed using a thermalcycler (My Cycler; BioRad Laboratories, Inc, Hercules, California). The reaction products were electrophoresed on 2% agarose gels, visualized with ethidium bromide, and examined by ultraviolet transillumination. Band intensities of reverse transcriptase–polymerase chain reaction (RT-PCR) products were quantified using ImageJ (Image Processing and Analysis in Java from http://rsb.info.nih.gov/ij/). Relative levels of mRNA were expressed as the ratio of signal intensity for the target genes relative to that for the housekeeping gene S16.

Statistical Analysis

All data are presented as mean ± standard error of the mean (SEM) in each group. For comparisons made along the time of incubation, repeated measures analysis of variance followed

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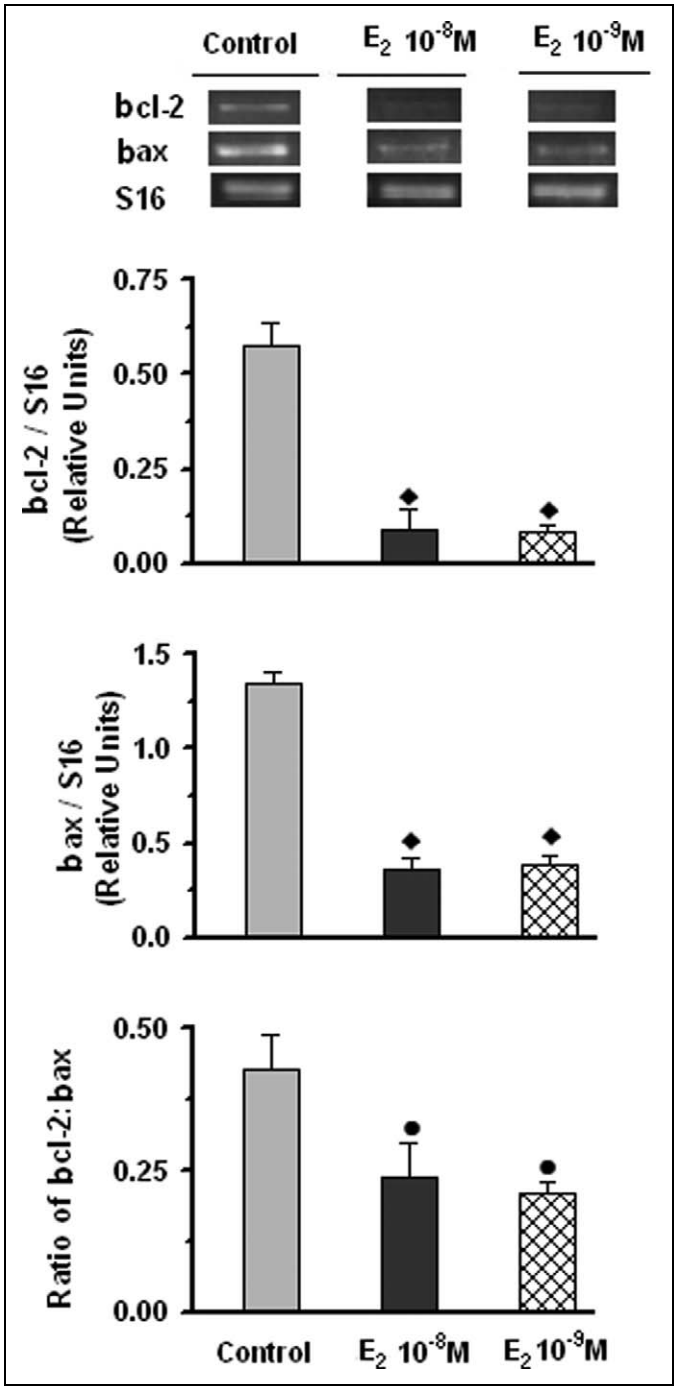

Figure 2. Direct effect of estradiol (E_2) on luteal mRNA expression of Bcl-2 and Bax in 21-day pregnant rats. The gel photographs were quantified using ImageJ software and expressed as arbitrary units. S16 was used as the housekeeping gene. Results are expressed as mean ± standard error of the mean ([SEM] n = 3). One-way analysis of variance followed by Tukey test was used. ♦$P < .001$, • $P < .005$.

by Tukey test was used. Instead, for multiple comparisons not involving repeated measures, 1-way analysis of variance followed by Tukey test was utilized. A value of $P < .05$ was considered statistically significant.

Results

Direct Effect of E_2 on Ovarian Progesterone Production and Luteal Physiology at the End of Pregnancy

To study whether E_2 has a direct effect on the release of progesterone from ovaries containing regressing corpora lutea on day 21 of pregnancy, whole ovaries were incubated in the presence of 10^{-6}, 10^{-8}, or 10^{-9} mol/L E_2. The release of progesterone, when assessed after 30, 60, 120, 180, or 240 minutes of incubation, declined significantly begining at 60 minutes of incubation, with respect to control groups (*$P < .05$; •$P < .005$; Figure 1A). Althouth the 3 doses of E_2 affected progesterone release, the lower concentrations appeared more efficient. Consequently, the remaining experiments were conducted using 10^{-8} or 10^{-9} mol/L E_2. The direct ovarian effect of E_2 was further studied by measuring the luteal mRNA expression of 3β-HSD involved in progesterone biosynthesis and 20α-HSD involved in progesterone catabolism, as well as the apoptotic regulators Bcl-2 and Bax. Estradiol at both 10^{-8} and 10^{-9} mol/L concentration reduced luteal expression of 3β-HSD (*$P < .05$) without altering 20α-HSD mRNA levels, when compared to controls receiving vehicle (Figure 1B). Messenger RNA levels of Bcl-2 and Bax were reduced by E_2 (♦$P < .001$), whereas the ratio Bcl-2 to Bax also declined when compared to controls groups (•$P < .005$; Figure 2).

Neural-Mediated Effect of Ganglionic E_2 on Ovarian Progesterone Production and Luteal Physiology at the End of Pregnancy

To examine whether E_2 on the CG had an effect on the synthesis and release of progesterone from ovaries containing regressing corpora lutea on day 21 of pregnancy, CG-SON-OV systems were incubated in the presence of 10^{-6}, 10^{-8}, or 10^{-9} mol/L E_2 in the ganglion compartment for 30, 60, 120, 180, and 240 minutes. At the end of the incubation periods, the concentration of progesterone was assessed in the ovarian compartment. The presence of 10^{-6} mol/L E_2 in the ganglion compartment decreased the release of ovarian progesterone only at 30 and 60 minutes, whereas 10^{-8} mol/L E_2 decreased progesterone release after 30, 60 and 180 minutes, whereas 10^{-9} mol/L E_2 in the ganglion decreased progesterone release at all the studied times when compared with control groups (*$P < .05$; Figure 3A). Ganglionic E_2 of 10^{-8} and 10^{-9} mol/L caused a decrease, yet not statisticaly significant, in 3β-HSD mRNA levels in the luteal tissue without modifying those of 20α-HSD (Figure 3B). Luteal expression of Bcl-2 and Bax, as well as the Bcl-2to-Bax ratio were not modified (Figure 4).

Discussion

In the present work, we examined the effect on luteal function after the addition of various concentrations of E_2 directly to an

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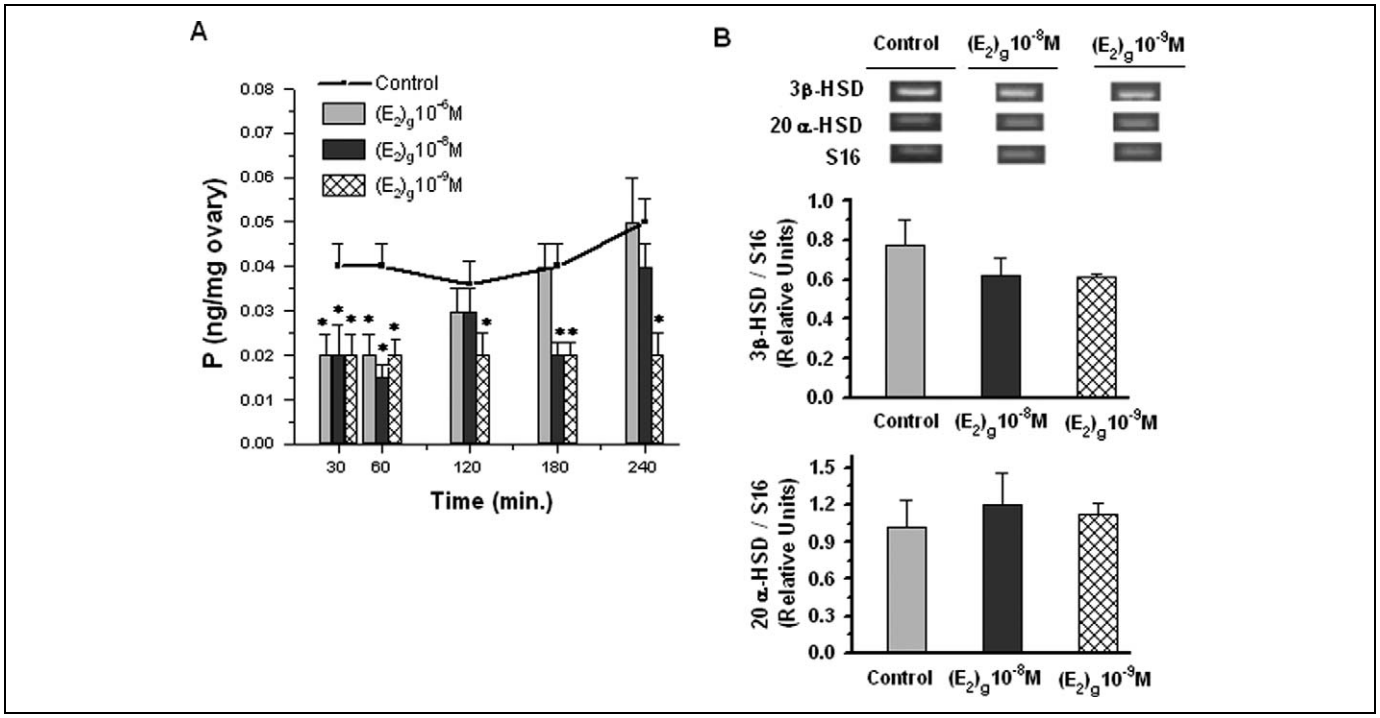

Figure 3. A, Ganglionic effect of estradiol ($[E_2]_g$) in the celiac ganglion–superior ovarian nerve–ovary system (CG-SON-OV) on ovarian progesterone (P) released and expressed as ng/mg ovary in 21-day pregnant rats. Results are expressed as mean ± standard error of the mean (SEM) of 6 animals per group. One-way analysis of variance followed by Tukey test was used. *$P < .05$. B, Ganglionic effect of estradiol (E_2) in the CG-SON-OV system on the luteal mRNA expression of enzymes 3β-HSD and 20α-HSD in 21-day pregnant rats. The gel photographs were quantified using ImageJ software and expressed as arbitrary units. S16 was used as the housekeeping gene. Results are expressed as mean ± SEM (n = 3). 3β-HSD indicates 3β-hydroxysteroid dehydrogenase; 20α-HSD, 20α-hydroxysteroid dehydrogenase; mRNA, messenger RNA.

OV incubation or indirectly on the ganglion compartment using an ex vivo CG-SON-OV model system from day 21 pregnant rats.

Estradiol, at the various concentrations utilized directly in the ovarian incubation medium, led to reduced progesterone release. Likewise, monkey corpora lutea were sensitive to E_2 locally produced in association with the regression of the corpus luteum,[20] whereas E_2 was shown to promote apoptosis in human luteal cells.[21] Our results are in agreement with the fact that a decline in luteal progesterone production in the rat is essential for luteal regression and parturition to occur. However, the effect of E_2 on luteal function appears to be species dependent, as in rabbits the steroid protects the corpus luteum from apoptosis rather than promoting apoptosis.[22]

We further found that the luteolytic effect of E_2 on day 21 pregnant rat ovaries was associated with a decline in the progesterone biosynthetic capacity marked by reduced 3β-HSD) mRNA expression, which has been previously shown to be hormonally regulated.[12] The inhibition of 3β-HSD mRNA expression by E_2 was reported previously by others.[23] Since E_2 was not able to modify the mRNA expression levels of the progesterone-catabolizing enzyme, 20α-HSD, our data suggest that E_2 impairs luteal progesterone production by decreasing its synthesis. As luteal regression in the pregnant rat begins with a decline in the progesterone-producing capacity of the corpora lutea, followed by structural regression of the gland occurring via programmed cell death,[12] we studied whether E_2 modifies the expression of antiapoptotic *Bcl-2* and proapoptotic *Bax* genes that are critical regulators of cell survival and death, respectively.[24] Moreover, we assessed the Bcl-2–to-Bax ratio, which seems critically associated with luteal apoptosis or survival.[25] In our in vitro study, E_2 added to the ovarian incubation compartment caused a decline in both Bcl-2 and Bax, as well as in the Bcl-2-to-Bax ratio, suggesting that the equilibrium between these 2 molecules favors Bax abundance and apoptosis, in agreement with a previous report indicating that these genes can be regulated by sexual steroids.[26] Consistent with our data, when E_2 was administered to pregnant rats on days 7 and 14 of pregnancy, serum progesterone declined on day 15 of pregnancy, likely as a consequence of a uterine-mediated effect.[27] Furthermore, in another study, when E_2 was given to rats postpartum, it accelerated luteal regression through a mechanism that posibbly involves the secretion of pituitary prolactin.[28]

Anesetti and colleagues[29] demonstrated the presence of estrogen receptors (ERs) α and β in the body of CG neurons of prepubertal rats, suggesting that the CG is likely a target of E_2. Furthermore, in the superior mesenteric ganglia of rats in oestrus stage, we demonstrated, using immunohistochemistry, the presence of ER-α in the cytoplasm of neural somas, whereas ER-β immunoreactivity was not observed (Vega Orozco A, Daneri C, Anesseti G, Cabrera R, Sosa Z, Rastrilla AM. ''Involvement of the oestrogenic receptors in superior

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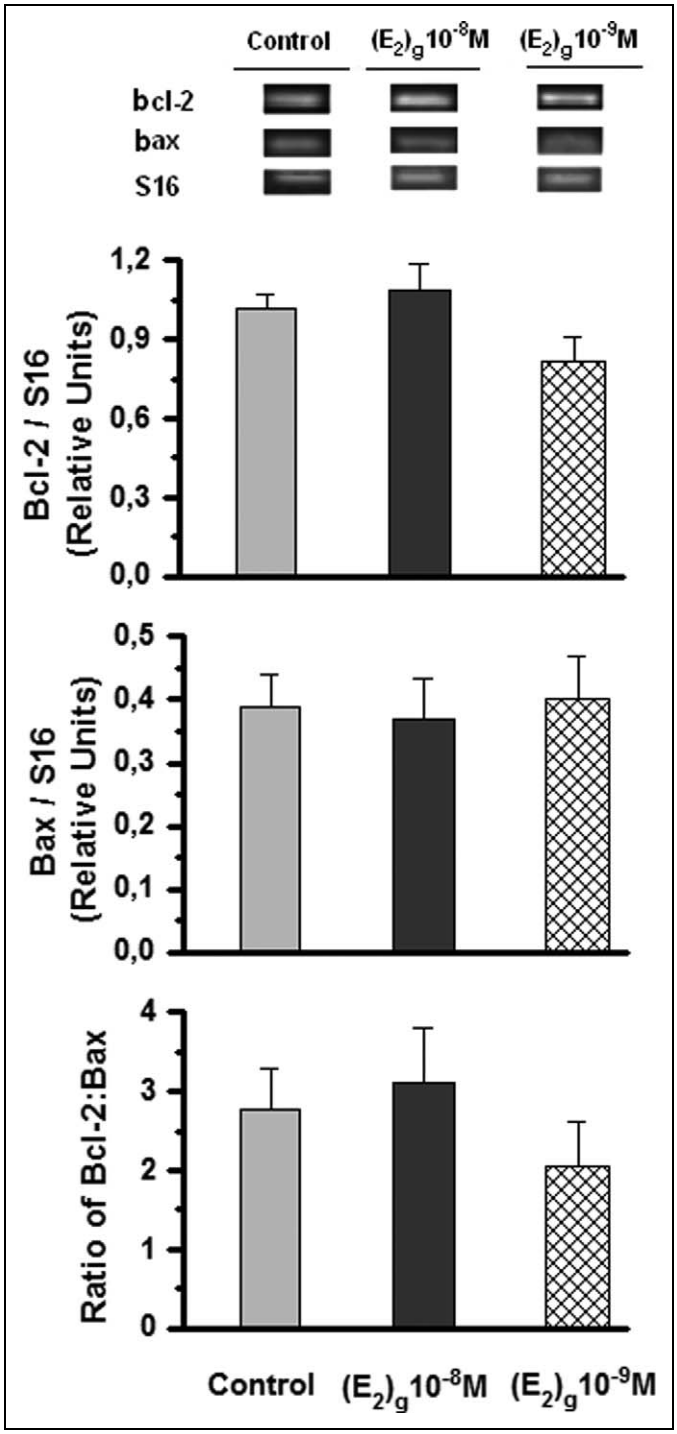

Figure 4. Ganglionic effect of estradiol ($[E_2]_g$) in the CG-SON-OV system on the luteal mRNA expression of antiapoptotic Bcl-2 and proapoptotic Bax in 21-day pregnant rats. The gel photographs were quantified using ImageJ software and expressed as arbitrary units. S16 was used as the housekeeping gene. Results are expressed as mean ± SEM (n=3). CG-SON-OV indicates celiac ganglion–superior ovarian nerve–ovary; mRNA, messenger RNA; SEM, standard error of the mean.

mesenteric ganglion on the ovarian steroidogenesis in rat", unpublished data). Based on this information, we added E_2 to the ganglionic compartment of the ex vivo GC-SON-OV system and observed that progesterone release from the ovarian compartment significantly declined, yet E_2 did not cause major modifications in the luteal mRNA expression (this work) and protein activity[30] of the progesterone biosynthetic enzyme 3β-HSD or the progesterone catabolizing enzyme 20α-HSD, suggesting that the neurotransmitters traveling via the SON, which can be deregulated in response to ganglionic E_2, may affect a releasable pool of progesterone without affecting its synthesis and/or metabolism. The presence of a pool of progesterone ready to be released without the need for new synthesis has been previously reported in the corpora lutea of various mammalian species including rats.[31-34] In future experiments, we expect to reveal the potential neurotransmitters mediating the ganglionic effect of E_2 and leading to reduced progesterone release in the ovary.

We also investigated whether the action of E_2 in the CG impacts the expression of mRNA coding for Bcl-2 and Bax in the ovary. Estradiol in the CG did not modify the ovarian expression of Bcl-2 and Bax or the Bcl-2-to-Bax ratio. These results reinforces the conclusion that we reached in previous studies where we demonstrated that the indirect luteotropic action of the aromatizable androgen androstenedione via the SON[2,5,35] is an effect of the androgen per se, not mediated through its previous conversion into E_2.

Taken together, our results demonstrate that E_2 either directly impacting the ovary or indirectly impacting the CG, negative affects the progesterone-producing capacity of the rat ovary at the end of pregnancy before parturition. This antiesteroidogenic effect is more pronounced when E_2 is directly impacting the ovary rather than when E_2 impacts the CG.

Acknowledgments

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Casais M, Sosa ZY, Rastrilla AM, Aguado LI. Coeliac ganglion adrenergic activity modifies ovarian progesterone during pregnancy: its inter-relationship with LH. *J Endocrinol*. 2001; 170(3):575-584.
2. Casais M, Delgado SM, Sosa Z, Rastrilla AM. Involvement of the coeliac ganglion in the luteotrophic effect of androstenedione in late pregnant rats. *Reproduction*. 2006;131(2):361-368.

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3. Casais M, Delgado SM, Sosa Z, Rastrilla AM. Pregnancy in rats is modulated by ganglionic cholinergic action. *Reproduction*. 2006; 131(6):1151-1158.
4. Casais M, Delgado SM, Sosa Z, Telleria CM, Rastrilla AM. The celiac ganglion modulates LH-induced inhibition of androstenedione release in late pregnant rat ovaries. *Reprod Biol Endocrinol*. 2006;4:66.
5. Vallcaneras SS, Casais M, Delgado SM, et al. Androgen receptors in coeliac ganglion in late pregnant rat. *Steroids*. 2009;74(6): 526-534.
6. Klein CM, Burden HW. Anatomical localization of afferent and postganglionic sympathetic neurons innervating the rat ovary. *Neurosci Lett*. 1988;85(2):217-222.
7. Eranko O. Small intensely fluorescent (SIF) cells and nervous transmission in sympathetic ganglia. *Annu Rev Pharmacol Toxicol*. 1978;18:417-430.
8. Prud'homme MJ, Houdeau E, Serghini R, Tillet Y, Schemann M, Rousseau JP. Small intensely fluorescent cells of the rat paracervical ganglion synthesize adrenaline, receive afferent innervation from postganglionic cholinergic neurones, and contain muscarinic receptors. *Brain Res*. 1999;821(1):141-149.
9. Chau YP, Chien CL, Lu KS. The permeability of capillaries among the small granule-containing cells in rat superior cervical ganglia: an ultrastructural lanthanum tracer study. *Histol Histopathol*. 1991;6(2):261-268.
10. Dalsgaard CJ, Vincent SR, Hokfelt T, et al. Coexistence of cholecystokinin- and substance P-like peptides in neurons of the dorsal root ganglia of the rat. *Neurosci Lett*. 1982;33(2):159-163.
11. Cardinali DP, Vacas MI, Gejman PV, et al. The sympathetic superior cervical ganglia as "little neuroendocrine brains". *Acta Physiol Lat Am*. 1983;33(3):205-221.
12. Stocco C, Telleria C, Gibori G. The molecular control of corpus luteum formation, function, and regression. *Endocr Rev*. 2007; 28(1):117-149.
13. Guo K, Wolf V, Dharmarajan AM, et al. Apoptosis-associated gene expression in the corpus luteum of the rat. *Biol Reprod*. 1998;58(3):739-746.
14. Gibori G, Chen YD, Khan I, Azhar S, Reaven GM. Regulation of luteal cell lipoprotein receptors, sterol contents, and steroidogenesis by estradiol in the pregnant rat. *Endocrinology*. 1984;114(2):609-617.
15. McLean MP, Puryear TK, Khan I, et al. Estradiol regulation of sterol carrier protein-2 independent of cytochrome P450 side-chain cleavage expression in the rat corpus luteum. *Endocrinology*. 1989;125(3):1337-1344.
16. Shaikh AA. Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy. *Biol Reprod*. 1971;5(3):297-307.
17. Bussmann LE. Prostaglandin F-2 alpha receptors in corpora lutea of pregnant rats and relationship with induction of 20 alpha-hydroxysteroid dehydrogenase. *J Reprod Fertil*. 1989;85(2): 331-341.
18. Aguado LI. Role of the central and peripheral nervous system in the ovarian function. *Microsc Res Tech*. 2002;59(6):462-473.
19. Bussmann LE, Deis RP. Studies concerning the hormonal induction of lactogenesis by prostaglandin F2 alpha in pregnant rats. *J Steroid Biochem*. 1979;11(4):1485-1489.
20. Duffy DM, Chaffin CL, Stouffer RL. Expression of estrogen receptor alpha and beta in the rhesus monkey corpus luteum during the menstrual cycle: regulation by luteinizing hormone and progesterone. *Endocrinology*. 2000;141(5):1711-1717.
21. Vaskivuo TE, Tapanainen JS. Apoptosis in the human ovary. *Reprod Biomed Online*. 2003;6(1):24-35.
22. Goodman SB, Kugu K, Chen SH, et al. Estradiol-mediated suppression of apoptosis in the rabbit corpus luteum is associated with a shift in expression of bcl-2 family members favoring cellular survival. *Biol Reprod*. 1998;59(4):820-827.
23. Depp R, Cox DW, Pion RJ, Conrad SH, Heinrichs WL. Inhibition of the pregnenolone delta 5-3 beta hydroxysteroid dehydrogenase-delta 5-4 isomerase systems of human placenta and corpus luteum of pregnancy. *Gynecol Invest*. 1973;4(2):106-120.
24. Yin XM, Oltvai ZN, Korsmeyer SJ. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. *Nature*. 1994;369(6478):321-323.
25. Antonsson B. Bax and other pro-apoptotic Bcl-2 family "killer-proteins" and their victim the mitochondrion. *Cell Tissue Res*. 2001;306(3):347-361.
26. Gompel A, Somai S, Chaouat M, et al. Hormonal regulation of apoptosis in breast cells and tissues. *Steroids*. 2000;65(10-11): 593-598.
27. Tamura H. Role of the nongravid part of the uterus in the luteolytic effects of estrogen in pregnant rats. *Endocrinol Jpn*. 1983; 30(5):615-620.
28. Goyeneche AA, Telleria CM. Exogenous estradiol enhances apoptosis in regressing post-partum rat corpora lutea possibly mediated by prolactin. *Reprod Biol Endocrinol*. 2005; 3:40.
29. Anesetti G, Lombide P, Chavez-Genaro R. Prepubertal estrogen exposure modifies neurotrophin receptor expression in celiac neurons and alters ovarian innervation. *Auton Neurosci*. 2009; 145(1-2):35-43.
30. Campo Verde Arbocco F, Vallcaneras S, Casais M, Rastrilla AM. Influence of estradiol in the peripheral neural regulation of ovary at the end of pregnancy in the rat. *Biocell*. 2009;33(Suppl 1): A53-A98.
31. Bramley TA, Menzies GS. Subcellular fractionation of the porcine corpus luteum: sequestration of progesterone in a unique particulate fraction. *J Endocrinol*. 1988;117(3):341-354.
32. Bramley TA, Menzies GS. Particulate binding sites for steroid hormones in subcellular fractions of the ovine corpus luteum: properties and hormone specificity. *Mol Cell Endocrinol*. 1994; 103(1-2):39-48.
33. Menzies GS, Bramley TA. Specific binding sites for progesterone in subcellular fractions of the porcine corpus luteum. *J Endocrinol*. 1994;142(1):101-110.
34. Telleria CM, Stocco CO, Stati AO, et al. Dual regulation of luteal progesterone production by androstenedione during spontaneous and RU486-induced luteolysis in pregnant rats. *J Steroid Biochem Mol Biol*. 1995;55(3-4):385-393.
35. Vallcaneras SS, Casais M, Anzulovich AC, et al. Androstenedione acts on the coeliac ganglion and modulates luteal function via the superior ovarian nerve in the postpartum rat. *J Steroid Biochem Mol Biol*. 2011;125(3-5):243-250.

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