Inhibition of cytochrome P-450 C17 enzyme by a GnRH agonist in ovarian follicles from gonadotropin-stimulated rats

Griselda Irusta,1,8 Fernanda Parborell,1,8 and Marta Tesone1,2
1Instituto de Biología y Medicina Experimental-Consejo Nacional de Investigaciones Científicas y Técnicas; and
2Departamento de Química Biológica, Facultad de Ciencias Exactas, Universidad de Buenos Aires, Buenos Aires, Argentina
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Irusta G, Parborell F, Tesone M. Inhibition of cytochrome P-450 C17 enzyme by a GnRH agonist in ovarian follicles from gonadotropin-stimulated rats. Am J Physiol Endocrinol Metab 292: E1456–E1464, 2007; doi:10.1152/ajpendo.00226.2006.—Our objective was to study the direct action of a GnRH-I agonist, leuprolide acetate (LA), on ovarian steroidogenesis in preovulatory follicles obtained from equine chorionic gonadotropin (eCG)-treated rats. Previously, we have demonstrated an inhibitory effect of LA on steroidogenesis and follicular development. In this study, we tested the hypothesis that gonadotropin-releasing hormone (GnRH) exerts its negative effect on follicule development by inhibiting theca cytochrome P-450 C17 (P450C17) α-hydroxylase expression and, consequently, androgen synthesis. Studies were carried out in prepubertal female rats injected with either eCG (control) or eCG plus LA (LA) and killed at different time points. Immunohistochemical studies indicated that LA induced steroidogenic acute regulatory protein (StAR) expression mainly in theca cells of preantral and antral follicles. In addition, serum proges- terone levels increased significantly (P < 0.05), whereas those of androsterone decreased (P < 0.05) after 8 h of LA treatment. This inhibition caused by LA seemed to be a consequence of the decreased expression of follicular P450C17 α-hydroxylase, as demonstrated by Western blot and RT-PCR techniques. In vitro studies using follicles isolated from 48-h-eCG-treated rats and cultured with LA showed a significant (P < 0.05) inhibition of FSH-induced androsterone follicular content as well as P450C17 α-hydroxylase protein levels, as determined by Western analysis. However, LA increased StAR protein expression in these follicles without significant changes in P450scc enzyme levels. Taking all these findings into account, we suggest that GnRH-I exerts a direct inhibitory action on gonadotropin-induced follicular development by decreasing the temporal expression of the P450C17 enzyme and, consequently, androgen production, thus reducing the supply of estrogens available to developing follicles.

ovary; follicle; gonadotropin-releasing hormone agonist; steroidogenesis

THE SUPPRESSION OF GONADOTROPS exerted by the administration of gonadotropin-releasing hormone (GnRH) analogs on the pituitary-gonadal axis is well known. The agonists initially stimulate the secretion of large amounts of gonadotropins, followed by a desensitization of the pituitary and the subsequent inhibition of gonadal steroid production (33, 62). However, in the last several years there has been increasing evidence that GnRH is an intrasynaptic regulator factor. A GnRH-like peptide, GnRH receptors, and transcription products from their genes have been found in ovarian tissue (3, 7, 31, 65), and a direct inhibitory effect of this decapetide has been observed on steroidogenesis in both granulosa cells (Gc) (31, 32, 35, 38, 40, 43, 58) and in corpus luteum (1, 62, 64). In addition, we (57) have demonstrated that in vitro treatment with a GnRH-I agonist (GnRH-I-a) produces an increase in apoptosis of prevulatory follicles by interfering with FSH, cAMP, and/or growth factors.

Our recent studies in prepubertal gonadotropin-stimulated rats have shown changes in the follicular expression of steroidogenic acute regulatory protein (StAR), a protein involved in the transfer of cholesterol from the outer to the inner mitochondrial membrane. In particular, 48 h of chronic in vivo GnRH-I-a treatment increases follicular concentrations of progesterone due to the enhancement of StAR expression and causes a decrease in follicular and serum concentrations of androgens and estradiol (34). Because estradiol is essential for normal follicular growth and atresia rescue (29), the previously observed GnRH-induced inhibition of folliculogenesis and increase in apoptosis appear to take place through alterations in the ovarian steroid pathway (1, 49, 56, 57).

Cytochrome P-450 C17 (P450C17) is a key branch point enzyme in the pathways of steroid hormone biosynthesis. It is associated with the smooth endoplasmic reticulum (SER) and catalyzes two sequential reactions: the hydroxylation of the C21 steroids progesterone or pregnenolone (17α-hydroxylase activity) and the cleavage of the two-carbon side chain (C17,20-lyase activity) to yield the C19 steroids androstenedione or dehydroepiandrosterone, respectively (19). Thus, this enzyme, confined to theca cells, is a crucial factor for the proper supply of androgens that are used as substrates for estrogen synthesis. Moreover, the P450C17 enzyme is considered a theca cell marker (23). The enzymes 5α-reductase and 3β-hydroxysteroid dehydrogenase, also localized in SER, are responsible for the reduction and conversion of androstenedione to testosterone and 5α-androstane-3α,5β-diol, respectively.

Available data indicate that thecal P450C17 expression is cAMP dependent in humans, cattle (14, 68), rats (21, 27), and pigs (9). LH appears to be essential for the maintenance of thecal cell P450C17 expression in all mammals studied to date (13). Many additional factors such as activin, inhibin, and insulin-like growth factor I also seem to regulate this enzyme (72).

There are several lines of evidence related to the direct effect of GnRH on gonadal androgen biosynthesis. Inhibition of testicular testosterone secretion in rats and toads has been reported (6, 69). Magoffin, Erickson, and colleagues (18, 48) have reported that GnRH treatment reduces basal and hCG-stimulated 17α-hydroxylase and C17,20-lyase in cultures of...
ovarian interstitial cells obtained from hypophysectomized immature rats. However, despite the increasing evidence of a GnRH effect on P450C17 activity in ovarian theca cells, little is known about the regulation of this enzyme expression at the protein level. Until now, no studies have been performed to establish whether GnRH agonists directly interfere with gonadotropin actions on P450C17 mRNA and protein expression in the ovary.

In the present report, we studied the role of GnRH in gonadotropin-stimulated prepubertal rats injected with either equine chorionic gonadotropin (eCG; control) or eCG plus GnRH-I-a [leuprolyl aceta (LA)] and killed at different time points. Specifically, we 1) expanded our earlier study of StAR protein regulation to include its immunohistochemical localization in ovarian cells and 2) examined changes of follicular P450C17 expression and serum steroid levels. Additionally, we determined 3) in vitro effect of GnRH-I-a on androsterone content, StAR, P450C17, and P450sc mRNA expression in FSH-stimulated follicles.

MATERIALS AND METHODS

Materials. LA, a GnRH-I-a, was obtained from Abbott Laboratories (Buenos Aires, Argentina). The original ampoule (2.8 mg/5 ml) was dissolved in saline to obtain the appropriate concentration. Syntex (Buenos Aires, Argentina) provided the eCG (Novormon). HEPES, SDS, and anti-rabbit secondary antibody conjugated with horseradish peroxidase were obtained from Sigma Chemical (St. Louis, MO). [1,2-3H(N)]progesterone (P4-3H) and [9,11-3H(N)]androsterone (Buenos Aires, Argentina) provided the eCG (Novormon). HEPES, SDS, and anti-rabbit secondary antibody conjugated with horseradish peroxidase were obtained from Sigma Chemical (St. Louis, MO). Dulbecco’s modified Eagle medium (DMEM; 4.5 g glucose/l), Ham’s F-12 nutrient mixture (F-12), fungizone (250 μg/ml), and gentamicin (10 mg/ml) were purchased from Invitrogen (Carlsbad, CA). All other chemicals were of reagent grade from standard commercial sources. StAR polyclonal rabbit antiserum was raised against a peptide fragment (amino acids 88--98) of the murine StAR sequence and was kindly donated by Dr. D. M. Stocco (Texas Tech University Health Sciences Center) (10). Rabbit antibody against bovine P450C17 was a kind gift from Dr. Anita Payne (Stanford University Medical Center, Stanford, CA). Rabbit-anti-porcine P450C17 antisera was a generous gift from Dr. D. B. Hales (University of Illinois).

GnRH agonist treatment. Immature female 23- to 25-day-old Sprague-Dawley rats were allowed food and water ad libitum and kept at room temperature at a range of 21–23°C with a 12:12-h light-dark cycle. The animals were killed by CO2 asphyxiation or decapitated at room temperature at a range of 21–23°C with a 12:12-h light-dark cycle. The original ampoule (2.8 mg/5 ml) was dissolved in saline to obtain the appropriate concentration. Syntex (Buenos Aires, Argentina) provided the eCG (Novormon). HEPES, SDS, and anti-rabbit secondary antibody conjugated with horseradish peroxidase were obtained from Sigma Chemical (St. Louis, MO). Dulbecco’s modified Eagle medium (DMEM; 4.5 g glucose/l), Ham’s F-12 nutrient mixture (F-12), fungizone (250 μg/ml), and gentamicin (10 mg/ml) were purchased from Invitrogen (Carlsbad, CA). All other chemicals were of reagent grade from standard commercial sources. StAR polyclonal rabbit antiserum was raised against a peptide fragment (amino acids 88--98) of the murine StAR sequence and was kindly donated by Dr. D. M. Stocco (Texas Tech University Health Sciences Center) (10). Rabbit antibody against bovine P450C17 was a kind gift from Dr. Anita Payne (Stanford University Medical Center, Stanford, CA). Rabbit-anti-porcine P450C17 antisera was a generous gift from Dr. D. B. Hales (University of Illinois).

RT-PCR analysis. Total RNA was extracted from control and LA groups (100 follicles/group) with Trizol reagent (Invitrogen) according to the manufacturer’s instructions. For amplification of the rat P450C17 cDNA, the designed primers used were the sense primer 5'-AAGTTGCTGTCCTGGTTT-3' and the antisense primer 5'-GGACTGACTGCTGGTGGC-3'. β-Actin cDNA was amplified as an internal control using a commercial set of primers (the sense primer 5'-CGGAAAGCTTGGTGGTGGC-3' and the antisense primer 5'-ACCCACACTGTGCCCATCTA-3'). β-Actin mRNA has been found to be expressed at a constant level independent of follicle status and size. In addition, its expression is not affected by growth factors or gonadotropins (42, 70). The molecular product sizes of the target genes (P450C17 and β-actin) were ~647 bp and 289 bp, respectively. PCR for P450C17 and β-actin were run separately, and conditions for each set of primers were optimized. Validation of the RT-PCR assay was performed, testing different numbers of cycles to ensure semiquantification of the product in the linear phase of the amplification. Briefly, 1 μg of total RNA obtained from the different groups was treated with 1 μg of DNaseA (Invitrogen). RT reaction was carried out at 37°C for 50 min in a 20-μl reaction volume using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) and oligo primers (Biodynamics). PCR consisted of denaturation at 94°C for 1.5 min, annealing at 60°C for 30 s, and extension at 72°C for 2 min for 25–30 cycles total. A final cycle of extension at 72°C for 5 min was included. The optimized number of PCR cycles (linear phase) used were 30 (P450C17 and β-actin) and 25 (β-actin, respectively). P450C17 and β-actin PCR products were separated on a 2% agarose gel that was stained with ethidium bromide for 15 min to enhance sensitivity. Gels were visualized on a UV transilluminator and photographed, and the bands for P450C17 and β-actin were quantified using the appropriate software (Scion Image). The PCR products were purified and sequenced at the ONPRC Molecular and Cell Biology Core facility using an ABI 3100 automated sequencer to confirm identity.

Western Blot for StAR, P450scx, and cytochrome P450C17. Sixty healthy AF obtained from the control and LA-treated groups were homogenized at 4°C in 500 μl of lysis buffer (20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 1% Nonidet P-40, and 10% glycerol) with protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, 0.025 mM N-ethylmaleimide, 0.025 mM N-ethylmaleimide, and 0.025 mM N-ethylmaleimide). The homogenate were centrifuged at 4°C for 10 min at 10,000 × g, and protein concentration in the supernatant was determined by Bradford assay. Equal amounts of
proteins (40 μg) were boiled for 5 min and resolved by 10% SDS-polyacrylamide gel at 25 mA for 2 h. The proteins were transferred for 2 h onto nitrocellulose membranes (Amersham Biosciences) in transfer buffer containing 20% methanol (vol/vol), 0.19 M glycine, and 0.025 M Tris base (pH 8.3) at 4°C. Blots were then blocked at 4°C in TBS (4 mM Tris·HCl, pH 7.5, 100 mM NaCl) containing low-fat powdered milk (5%) and Tween-20 (0.2%). Rabbit polyclonal anti-StAR (1:2,000), anti-P450scC (1:2,000), and anti-P450C17 (1:100) were used as primary antibodies. Protein bands were detected by incubation with anti-rabbit IgG (1:1,000 dilution, 1 h) conjugated to horseradish peroxidase (Sigma). β-Actin was detected with anti-
mouse (1:1,000 dilution, 1 h) secondary antibody (R&D Systems). Reactive protein bands were visualized by chemiluminescence, using the manufacturer’s protocol (Amersham Pharmacia). Negative controls were obtained in the absence of the primary antibody. The levels of protein were compared in extracts from the control and LA groups and analyzed by densitometric studies. Optical density data are expressed as arbitrary units ± SE (n = 3). In the in vitro experiments, the proper loading was evaluated by staining the membranes with Ponceau S. As an internal control, the density of each protein was normalized to the density of a band that was observed on the protein transference pattern in all Ponceau S-stained membranes. This band was selected since it remained unchanged under different treatments. On the other hand, for the in vitro experiments, the density in each band was normalized using β-actin (mouse monoclonal, Abcam) as an internal control. The Ponceau S method was validated in selected samples using β-actin as control loading, since it was observed that the ratio of the desired protein band against β-actin band was similar to the ratio obtained when the normalization was performed against the Ponceau S band (data not shown).

Culture of isolated ovarian follicles. To study the in vitro effects of LA on ovarian steroidogenic proteins, AF were dissected from ovaries collected following eCG treatment and cleansed of adhering tissue in culture medium. The culture was initiated within 1 h of ovary removal, and the follicular pools (containing 60 follicles/sample) were incubated in triplicate under serum-free conditions at 37°C in 1 ml DMEM-F-12 (1:1 vol/vol), containing 10 mM HEPES, supplemented with fungizone (250 μg/ml) and gentamicin (10 mg/ml). Follicles were incubated for 2, 8, 12, 24, and 48 h with FSH (20 ng/ml) in either the presence or the absence of LA (100 ng/ml) at 37°C under 95% O2 conditions, the intra-assay and interassay variations were 8.0 and 14.2% for progesterone and 8.1 and 14.5% for androsterone, respectively.

Data analysis. All experiments were repeated three times with five animals per group, and incubations were performed in triplicate using follicle pools. Data regarding densitometric analysis and in vitro experiments were expressed as means ± SE of pooled results obtained from three independent experiments (n = 3). Statistical analyses were performed using two-way ANOVA for experiments including two variables, such as time and treatments. When values were significant, ANOVA analysis was followed by either Tukey’s or Bonferroni’s multiple comparison test. Values of P < 0.05 were considered significant.

RESULTS

GnRH-I-a INHIBITS OVARIAN CYTOCHROME P450C17 ENZYME

**GnRH-I-a effects on StAR expression.** To determine the cell type-specific expression of StAR protein in prepubertal rat ovaries, immunohistochemistry was performed on ovarian sections derived from eCG- (control group) and eCG plus LA (LA group)-treated animals. When these animals were killed 2 or 8 h after the eCG injection (Fig. 1, A and B, respectively), immunostaining for StAR was observed in theca cells (Tc) and stroma (st) of preantral follicles (PF) and AF after only 8 h of eCG administration. In contrast, Gc showed no expression of this protein. After 8 h of eCG plus LA administration, the expression of StAR was increased in Tc of either PF or AF (Fig. 1D) compared with either the 2-h eCG plus LA treatment (Fig. 1C) or the 8-h control group (Fig. 1B). In addition, Gc of AF exhibited a weak immunostaining for StAR protein after 8 h of eCG plus LA treatment (Fig. 1D), whereas no positive staining was detected in the same type of cells in PF.

Surprisingly, after 8 h of LA treatment, the theca layer in PF was thicker than that observed in ovarian sections from eCG-treated rats (Fig. 1, A vs. D).

In agreement with previous studies performed by Ronen-Fuhrmann et al. (61), the expression of StAR was not detected at time 0 (without treatment; data not shown).

**Effects of GnRH-I-a on serum progesterone and androsterone levels.** To determine whether the increase in ovarian StAR expression after the agonist treatment was correlated with steroid levels, we performed RIA in serum collected from both eCG- and eCG plus LA-treated animals. As shown in Fig. 2A, serum levels of progesterone increased significantly (P < 0.05) after 8 h of eCG plus LA administration but were not different from the corresponding control at other time points (2, 24, and 48 h). In addition, we analyzed serum levels of androsterone, the principal androgen produced by prepubertal rat ovaries (18). Figure 2B illustrates serum androsterone levels at different periods of treatment. The injection of eCG (control group) produced a time-dependent stimulation in androsterone production. Cotreatment with LA significantly inhibited eCG-stimulated androsterone production by 8 h (P < 0.05) of treatment.

**In vivo effect of LA on ovarian cytochrome P450C17 gene and protein expression.** To determine whether the decrease in serum androsterone by LA treatment was due to a decrease in P450C17 enzyme levels, we performed Western blot analyses of follicles obtained from the eCG- and eCG plus LA-treated animals (Fig. 3A). As shown, the GnRH-I-a treatment significantly decreased the relative intensity of P450C17 in the LA group compared with the control group at 8 h. To establish whether P450C17 regulation by the agonist also occurred at the mRNA level, we carried out RT-PCR analyses at all the time points mentioned. Figure 3B shows that mRNA levels...
were decreased after 8 h of eCG plus LA treatment, similar to the results observed with protein expression.

**Effect of LA on follicular androsterone in ovarian follicle cultures.** Ovarian follicles isolated from 48-h-eCG-treated rats were cultured with FSH or FSH plus LA for 8, 12, 24, and 48 h. This model has the advantage of keeping the integrity of the follicle and is currently used to investigate the pathways that control steroidogenesis, apoptosis, and follicular atresia (57, 66). By means of this model, we expected to analyze steroidogenesis regulation by the GnRH-I-a and to confirm whether the agonist affects this process directly. As shown in Fig. 4, follicular androsterone content was significantly decreased (P < 0.05) when follicles were coincubated for 24 h with FSH plus LA (Fig. 4).

**In vitro effect of LA on ovarian StAR, P450scc, and P450C17 protein expression.** To study the direct effects of LA on the expression of ovarian steroidogenic proteins, AF obtained from ovaries of eCG-stimulated rats were cultured for different periods of time (2, 8, 12, 24, and 48 h) in serum-free medium with FSH (20 ng/ml) in either the presence or absence...
of LA (100 ng/ml) (Fig. 5). Follicular content of StAR, P450sc, and P450C17 were determined by Western blotting. The levels of StAR detected in protein extracts obtained from LA-incubated follicles increased significantly at 12 h (FSH: 0.23 ± 0.09, FSH + LA: 1.67 ± 0.15, P < 0.05; Fig. 5A). This

protein was not detected after 24 and 48 h of incubation in FSH-incubated follicles in either the presence or absence of LA. No changes were observed in P450sc levels at the different periods of time studied (data not shown). Interestingly, follicles incubated with FSH plus LA showed a significant decrease in P450C17 protein expression at 12 h when compared with follicles incubated with FSH alone (FSH: 0.51 ± 0.04, FSH + LA: 0.16 ± 0.03, P < 0.05; Fig. 5B).

DISCUSSION

The results of the present study demonstrate, for the first time, that GnRH-I-a administered in vivo to gonadotropin-treated prepubertal rats induces an increase in StAR protein and a decrease in P450C17 expression and, consequently, in androgen production. The demonstration of this paradoxical effect in steroidogenic proteins and enzymes is novel. These findings were supported by in vitro studies confirming a direct action of GnRH on follicular steroidogenesis.

StAR is especially involved in the acute regulation of steroid synthesis in response to the gonadotropin surge. Ronen-Fuhrmann et al. (61) have demonstrated a biphasic increase in the levels of this protein after eCG administration in ovarian homogenates from immature rats. In this regard, we (34) have previously shown an increase in StAR protein content and messenger levels in healthy antral or preovulatory follicles from superovulated LA-treated rats after either acute or chronic treatment. These observations led us to study the time-dependent effect of a GnRH-I-a on steroidogenic proteins and steroid production.

In the present study, we demonstrated by immunohistochemical studies that the administration of a GnRH-I-a (LA) to gonadotropin-treated prepubertal rats produces an increase in ovarian StAR expression localized in theca cells of preantral and antral follicles. The results obtained in eCG-treated rats were in agreement with previous reports (61) in which a bright pattern of StAR staining in interstitial and theca cells was observed after gonadotropin treatment without expression of this protein in granulosa cells. However, in our study, after
cotreatment with LA, a weak StAR staining in granulosa cells was observed. In addition, when we quantified serum levels of progesterone, the increase of this progestagen in eCG plus LA-treated rats was parallel to StAR expression. In contrast, Yang et al. (73) have recently reported an inhibition in serum progesterone production in pregnant rats treated with a GnRH agonist for 8 h. In that study, the agonist was continuously administered and a higher dose was used. In addition, GnRH agonists stimulate progesterone accumulation in granulosa cells from Graffian follicles and plasma progesterone in acutely hypophysectomized rats (17, 28).

These reports indicate the existence of different responses to GnRH depending on the ovarian cell types and dose and route of GnRH administration. In antral follicles, a failure to express aromatase in granulosa cells is characterized by a high androgen-to-estrogen ratio in follicular fluid, an event that causes follicular atresia. Therefore, the accurate supply of androgens is critical for normal folliculogenesis beyond the antral stage (20). Furthermore, estrogen is necessary for both normal folliculogenesis and maintenance of the female phenotype of the somatic cells within the ovaries (20). Given the importance of androgens as follicular estrogen substrates (4, 12, 26, 44), we studied plasma androsterone levels in animals treated with the eCG plus GnRH-I-a, since this androgen has been reported to be one of the main 5α-metabolites in the immature rat ovary (47). In our experimental model, GnRH-I produced a marked decrease in serum androsterone in animals killed 8 h after the administration of the agonist. Our observations of the steroidogenic profile of the GnRH-I-treated animals support that the inhibition of the P450C17 enzyme accounts for the decreased androgen synthesis despite the increase in serum progesterone levels produced as a consequence of the increase of StAR expression. In this sense, it has been suggested that GnRH switches the interstitial cells from androgen- to progesterone-secreting cells (18, 46).

We also demonstrated in this work a negative effect on P450C17 follicular expression at messenger and protein levels caused by in vivo treatment with a GnRH agonist in gonadotropin-treated rats. Although 17α-hydroxylase/C17,20 lyase activity has been determined under different conditions, both in vivo and in vitro (15, 16, 39, 45, 51, 53), until now, few studies have been carried out on the expression of this enzyme at the protein level. In this regard, the relevance of our work resides in the correlation of P450C17 mRNA and protein expression levels and its regulation by GnRH-I. This is the first in vivo study in which different regulation points of P450C17 have been investigated in prepubertal gonadotropin-stimulated rats treated with a GnRH-I-a.

In addition, to elucidate whether the LA effect was due to a direct effect on ovarian cells and to discard a possible action of the agonist at the pituitary level, in vitro experiments were performed. These studies showed a significant inhibition of the FSH-induced follicular androsterone content in follicles incubated for 24 h with FSH plus GnRH-I-a. This effect may be a consequence of the direct effect of the agonist on P450C17 enzyme expression. In agreement with this hypothesis, blotting analyses showed a decrease in the P450C17 follicular expression after 12 h of culture in the presence of LA. Similarly to the in vivo effect of LA on StAR expression, the agonist also induced an increase in this protein after 12 h of incubation. Furthermore, in agreement with our previous in vivo results (34), no changes in P450scc protein expression were detected after in vitro LA follicle treatment.

There are several lines of evidence about GnRH-like peptides playing autocrine or paracrine regulatory roles in the ovary. It has been reported that an intrinsic GnRH system, with endogenous GnRH, GnRH receptor, and biological response, exists in rodent (2, 24, 52) and human (8, 11, 22, 30, 36, 37, 59) ovaries. It is well documented that GnRH-I possesses anti-
nadotropic effect in the rat ovary, downregulating the expression of FSH and LH receptors (67, 60), inhibiting gonadotropin-stimulated cAMP production (54, 41), and suppressing steroidogenic enzymes (32, 63). In addition, we demonstrated that GnRH-I treatment interferes with follicular recruitment, growth, and luteinization induced by gonadotropins (1, 25, 30). Moreover, we demonstrated that the GnRH antagonist treatment of gonadotropin-treated rats improves follicular development, suggesting a binding impairment of the GnRH-like peptide synthesized in the ovary to its receptors (1, 49, 56, 57). Considering all these findings, we propose a possible mechanism by which GnRH-I exerts a direct inhibitory action on follicular development through the alteration of key steroidogenic proteins and their products. These lines of evidence, together with our previous reports demonstrating that antral follicles from gonadotropin-treated rats are more sensitive toward undergoing an apoptotic process when they have been exposed to a GnRH-I treatment (55, 56), suggest that GnRH could act as an intraovarian factor by interfering with gonadotropin induction of follicular development.

In summary, we suggest that GnRH-I exerts a direct inhibitory action on gonadotropin-induced follicular development by causing a decrease in the temporal expression of the P450C17 enzyme and consequently in androgen production, thus reducing the supply of estrogens available to the developing follicles.

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