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Hormonal Regulation of GnRH and LHβ mRNA Expression in Cultured Rat Granulosa Cells

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Abstract We have recently demonstrated that the rat ovary expresses LHβ, FSHβ, and the common alpha subunit mRNA. In the present report, we studied the regulation of LHβ and of gonadotropin-releasing hormone (GnRH) mRNA expression in granulosa cells that were isolated from immature rats treated with either estrogen or pregnant mare serum gonadotropin (PMSG). In both cell types, GnRH agonist treatment resulted in a decrease in LHβ mRNA expression. However, only in cells derived from PMSG-treated rats, GnRH treatment produced an increase in GnRH mRNA expression. A markedly increased GnRH mRNA expression was also obtained in granulosa cells derived from PMSG-primed rats in response to LH. In addition, FSH reduced the expression of LHβ mRNA in granulosa cells from estrogen-primed rats. These results suggest that the expression of LHβ in the ovary is regulated by locally produced GnRH and by FSH from either the ovary or the pituitary.

Keywords GnRH/LHβ mRNA · Rat granulosa cells

Introduction

Ovulation is controlled by the hypothalamus that secretes gonadotropin-releasing hormone (GnRH) into the hypothalamic–pituitary portal blood system that reaches the pituitary gland and induces the secretion of the gonadotropic hormones, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). GnRH is secreted in pulses that change in frequency and magnitude during the estrous cycle. A GnRH surge occurs on the afternoon of proestrus, and induces a massive release of gonadotropic hormones, FSH and LH, from the pituitary, which leads in turn to ovulation.

GnRH, which is mainly produced by the hypothalamus, is also expressed in some peripheral organs, including the ovary (Oikawa et al. 1990), testes (Swerdloff et al. 1984; Bahk et al. 1995), and the pituitary gland (Bauer et al. 1981). The GnRH receptor is also expressed in these tissues (Pieper et al. 1981; Jones et al. 1980; Clayton et al. 1992; Whitelaw et al. 1995; Clayton et al. 1980; Bahk et al. 1995; Reeves et al. 1980). As only minute amounts of the hypothalamic GnRH are secreted directly into the pituitary portal system, and since GnRH is rapidly degraded (Koch et al. 1974) and vastly diluted in the general circulation, the effects of the hypothalamic GnRH are apparently restricted to the pituitary gland. Thus, the effects of GnRH on the ovary represent the response to a locally produced hormone.

Indeed, GnRH was found to induce ovulation in hypophysectomized rats (Corbin and Bex 1981; Bex and Corbin 1984; Ekholm et al. 1981), and a GnRH antagonist blocked the GnRH-, but not the LH-, induced ovulation in hypophysectomized rats (Dekel et al. 1983). GnRH was also found to regulate ovarian steroidogenesis and apoptosis (Leung et al. 2003), follicular atresia (Billig et al. 1994), oocyte maturation, and prostaglandin production (Naor et al. 1984). Another indication that GnRH has an important role on ovarian functions is suggested by its unique expression pattern in the ovary, which is different from

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that in the hypothalamus or the pituitary gland, throughout the estrous cycle (Schirman-Hildesheim et al. 2005).

Similar to GnRH, the gonadotropic hormones, LH and FSH, are also produced not only by the pituitary but also in peripheral organs. Expression of these hormones was found in the human, rat and mouse testes (Wahlstrom et al. 1983; Hovatta et al. 1986; Zhang et al. 1995a; Zhang et al. 1995b) as well as in the gilthead seabream ovary (Wong and Zohar 2004). We have recently reported that the rat ovary contains locally produced LH and expresses both LHβ and FSHβ and the common-α subunit mRNAs (Schirman-Hildesheim et al. 2008). By demonstrating the presence of LH in the ovaries of hypophysectomized rats, we excluded the possibility of pituitary hormone contamination. Therefore, it seems that hormones involved in the regulation of reproduction may be present in all levels of the reproductive axis: the hypothalamus, pituitary, and ovary, suggesting that a local gonadal GnRH–gonadotropin axis may exist within the ovary. However, the physiological significance of the local GnRH–gonadotropin axis in the ovary is still unknown.

The rat ovarian and testicular LHβ mRNA, in contrast to the pituitary LHβ mRNA, contains a long 5′ untranslated region (Zhang et al. 1995b; Schirman-Hildesheim et al. 2008), suggesting a different transcription start site as well as a different mode of transcriptional regulation as compared to those of the pituitary gland. Therefore, in this study, we used rat granulosa cell cultures to investigate the regulation of ovarian LHβ in vitro. Two different models of granulosa cell culture are currently used to study the regulation of gene expression. The first model is of cells isolated from immature rats primed with estrogen. This treatment induces a vast proliferation of the granulosa cells within the follicle, but does not lead to the formation of preovulatory follicles (Orly et al. 1980). The second model is of granulosa cells, isolated from immature rats primed with pregnant mare serum gonadotropin (PMSG), which induces maturation of the follicles towards ovulation (Neal and Baker 1974; Park et al. 2001; Granot and Dekel 1994). Specifically, in addition to cell proliferation, this treatment induces expression of the LH receptors in the granulosa cells (Vidyashankar and Moudgal 1984). Without prior knowledge as to which granulosa cell model will be relevant for our studies, we compared the basal expression of both LHβ and GnRH mRNAs in both cell models. We found that the expression of LHβ mRNA was lower in granulosa cells isolated from PMSG-primed rats as compared to granulosa cells isolated from estrogen-primed rats. In addition, FSH treatment markedly reduced the expression of LHβ mRNA in granulosa cells from estrogen-primed rats. We also found that, in granulosa cell cultures derived from either PMSG- or estrogen-primed rats, GnRH agonist induced a decrease in LHβ mRNA expression.

Methods

Experimental Animals

Rats were purchased from Harlan Laboratories (Rehovot, Israel) and all experiments were carried out in compliance with the regulations of the Weizmann Institute of Science and using accepted standards of humane animal care. Animals were housed under constant conditions of temperature and humidity, with lights on between 6 A.M. and 8 P.M. Food and water were available ad libitum.

Estrogen Priming

Sexually immature, 23-day-old female Wistar rats were injected s.c. with 1 mg/ml estradiol in propylene glycol (Sigma, Rehovot, Israel) at 8:00 A.M. for three consecutive days to induce the proliferation of granulosa cells within the follicles (Orly et al. 1980). On the fourth day, the animals were euthanized and the ovaries were excised for granulosa cell isolation as described below.

PMSG Priming

Sexually immature female Wistar rats (23–24 days old) were injected s.c. with 10 IU of pregnant mare's serum gonadotropin (PMSG, Chrono-gest Intervet, Boxmeen, The Netherlands) at 10:00 A.M. to stimulate follicular development. Forty-eight hours afterwards, the animals were euthanized and the ovaries were excised for granulosa cell isolation as described below.

Isolation of Granulosa Cells

Animals were euthanized 72 h after the first estrogen administration or 48 h after PMSG administration; the ovaries were excised and transferred to Petri dishes containing L15 medium (Biological Industries, Beit Haamek, Israel). Follicles were punctured with a 271/2G needle and the granulosa cells and cumulus–oocytes complexes were gently squeezed out of the follicles. The oocytes were removed and the granulosa cells were collected after gentle centrifugation (500 rpm for 5 min) and suspended in a serum-free medium (Orly et al. 1980) containing DMEM:F15 medium supplemented with 2 μg/ml insulin (Biological Industries), 5 μg/ml transferin (Biological Industries), and 40 ng/ml hydrocortisone (Sigma). The cells were seeded in serum-coated 12-well plates (equivalent of one ovary/well) and grown at 37°C in a humidified incubator with 5% CO_2 atmosphere. On the next day, the medium was replaced with fresh media containing the desired treatments. Twenty-four hours after treatment, the medium was removed and cells were lysed

with TriReagent (Molecular Research Center, Inc., Cincinnati, OH, USA) for RNA extraction as described below.

Hormonal Treatments of Granulosa Cells

Granulosa cells in culture were treated with one of the following hormones, as indicated in the 'Results' section: 200 ng/ml of ovine-FSH (NIAMDD-oFSH-S16, N.I.H., Bethesda, MD, USA); 200 ng/ml of ovine-LH (NIAMDD-oLH-S18); 10 nM or 1 μM of D-Trp^{6}GnRH (Bachem AG, Bubendrof, Switzerland); 10 μM of the GnRH antagonist Cetrorelix (Asta Medica AG, Frankfurt, Germany).

RNA Purification and Reverse Transcriptase

Total RNA was extracted from granulosa cells by TriReagent (1 ml/well) as recommended by the manufacturer. To remove residual DNA, the RNA was treated with a TURBO DNA-free kit (Ambion, Austin, TX, USA) as recommended by the manufacturer. Equal amounts of RNA were reverse transcribed using 40 U of SuperScript II (Invitrogen Life Technologies, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions.

Real-time PCR

Primers for all genes were designed on two different exons so as to span one intronic sequence. The sequences used to amplify the GnRH, ribosomal protein L19, and cyclophillin A were described previously (Schirman-Hildesheim et al. 2005). The sequences used to amplify the LHβ gene (accession no. NM_001033975) were as follows: sense 5′ TCACCTTCACCACCAGCATC; antisense 5′ GACCCCA CAGTCAGAGCTA. These primers were used at the final concentration of 75 nM.

All real-time PCRs were carried out on a Rotor-Gene 3000 (Corbett Research, Sydney, Australia) using the Absolute QPCR Master Mix (ABgene, Surrey, UK) with SYBR Green-I. Reaction protocols had the following format: 15 min at 95°C for enzyme activation, followed by 40–50 cycles of 15 s at 95°C, 10 s at 60°C, and 20 s at 72°C, at the end of which fluorescence was measured (except for LH gene where fluorescence was measured at an additional 85°C step). At the end of the cycling protocol, a melt curve was produced, with continuous fluorescence measurement from 65 to 99°C. All reactions contained the same amount of cDNA, 10 μl Absolute QPCR Master Mix, primers diluted according to an optimized combination, to a final volume of 20 μl.

Each real-time PCR included a no-template control as well as five or six serial 4-fold dilutions, in duplicates, of a cDNA pool containing all experimental samples of the respective experiment. The prenormalized DNA quantity of each gene in every sample was estimated relative to this dilution series. This dilution series also served to assess the reaction performance (E and r^2). The threshold cycle (Ct) was set so as to obtain the highest reaction efficiency and correlation coefficient.

Data Analyses and Statistics

The relative amount of GnRH or LHβ mRNA in each sample was calculated by dividing the prenormalized DNA quantity of these genes, obtained from the dilution series (see above), by the geometric mean of the DNA quantities of the reference genes. This normalized DNA quantity is hereafter referred to as the relative expression level of GnRH or LHβ mRNA.

All statistical analyses were performed using JMP Statistical Discovery software (version 6, SAS Institute, Inc., Cary, NC, USA). The distribution of the relative amounts of GnRH and LHβ mRNA in all samples were tested for normality using the Kolmogorov–Smirnov–Lilliefor test. In all cases studied, the log-transformed data displayed a normal distribution ($P>0.05$). Statistical evaluation of differences between time groups was performed using both parametric and non-parametric multiple comparison tests (one-way ANOVA and the Wilcoxon rank-sum test, respectively), followed by pairwise comparisons of means using the least significant difference test (Tukey–Kramer HSD) at a confidence level of 95%. Student's t test at a confidence level of 95% was used to compare two groups.

Results

Comparison of the Basal Expression of LHβ and GnRH mRNAs in Two Models of Granulosa Cell Cultures

Two different models for granulosa cell culture (estrogen- or PMSG-primed immature rats) are currently used to study the regulation of gene expression. Without prior knowledge as to which granulosa cell model will be relevant for our inquiry, we compared the basal expression of both LHβ and GnRH mRNAs in both cell models. As shown in Fig. 1, both cell types express LHβ and GnRH mRNAs. However, the expression of LHβ and that of GnRH mRNAs is lower in granulosa cells isolated from PMSG-primed rats as compared to those from estrogen-primed rats.

The Effect of FSH on the Expression of LHβ and GnRH mRNAs in Granulosa Cells from Estrogen-primed Rats

To verify whether the difference in gene expression between the two granulosa cell models is due to the

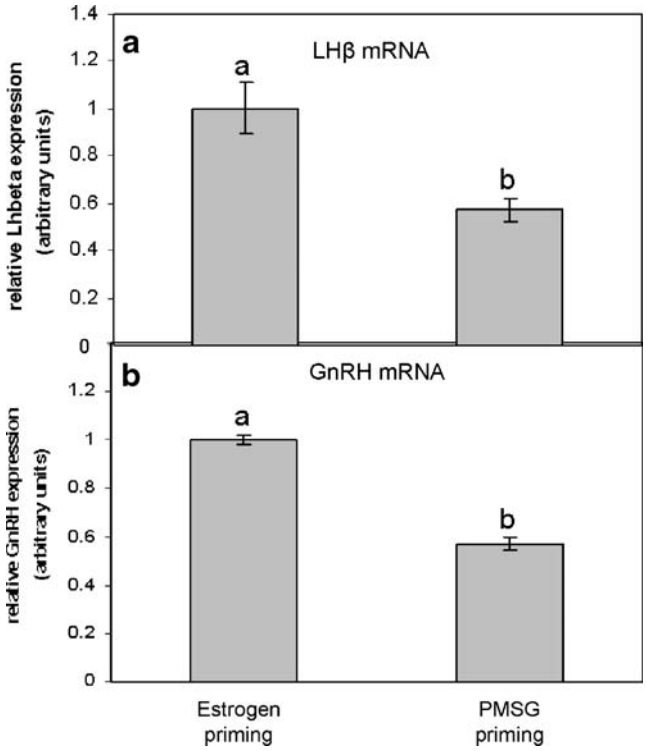

Figure 1 Relative expression of LHβ (**a**) and GnRH (**b**) mRNAs in granulosa cell culture produced from estrogen- or PMSG-primed immature female rats. Sexually immature 23-day-old female Wistar rats were injected s.c. either with 1 mg/ml estradiol in propylene glycol for three consecutive days or with a single administration of 10 IU of PMSG. Animals were euthanized 72 h after the first estrogen administration or 48 h after PMSG administration, and granulosa cells were isolated and grown in serum-free medium as described in the 'Methods' section. Two days later, cells were lysed and RNA was purified. Relative gene expression was quantified by real-time PCR as described in the 'Methods' section. Results from two experiments, each with triplicates or quadruplicates, are presented. *Bars* indicate SEM. *Different letters* indicate statistically significant differences between groups ($P<0.01$)

activation of FSH receptor by PMSG, we treated granulosa cells from estrogen-primed rats with 200 ng/ml of ovine-FSH. Figure 2 shows that FSH indeed caused a major decrease in the expression of LHβ mRNA, similar to that found following priming with PMSG (Fig. 1). However, the expression of GnRH mRNA was not affected. Thus, the lower rate of expression of GnRH mRNA in granulosa cells following PMSG priming seems not to be due to FSH receptor activation.

The Effect of LH on the Expression of LHβ and GnRH mRNA in Granulosa Cells from PMSG-primed Rats

PMSG can also activate the LH receptor (Vidyashankar and Moudgal 1984). In order to find out whether LH may regulate its own expression, we treated granulosa cells from PMSG-primed immature rats with 200 ng/ml ovine-LH. As shown in

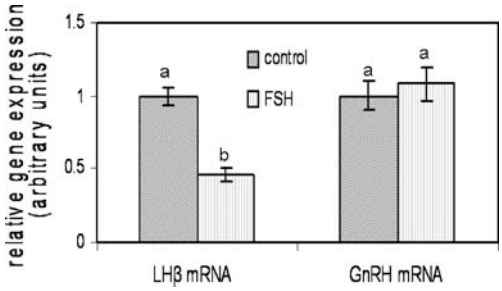

Figure 2 The effect of ovine-FSH on the expression of LHβ and GnRH mRNA in granulosa cells from estrogen-primed rats. Granulosa cells isolated from estrogen-primed rats were grown in serum-free medium. On the next day, the medium was replaced with ovine-FSH (200 ng/ml) containing medium for 24 h, thereafter RNA was extracted from the cells. The results are from two experiments, each with triplicates or quadruplicates. *Bars* indicate SEM. *Different letters* indicate statistically significant differences between control and treatment ($P<0.005$)

Fig. 3, the expression of LHβ mRNA was not affected by LH. However, LH treatment caused an 80% increase in the expression of the GnRH mRNA. Thus, the findings that were demonstrated in Fig. 1, demonstrating that granulosa cells from rats primed with PMSG express less GnRH mRNA as compared to cells isolated from rats primed with estrogen, cannot be attributed to the LH receptor signaling.

The Effect of GnRH Analogs on LHβ and GnRH mRNA Expression in Granulosa Cell Cultures from Estrogen- or PMSG-primed Rats

GnRH is known to regulate the expression of LHβ in the pituitary gland (Andrews et al. 1988; Lalloz et al. 1988). Therefore, we investigated whether GnRH also regulates LHβ expression in granulosa cells. Since GnRH and its receptor are expressed in granulosa cells, we also examined the possibility that a GnRH antagonist will elicit a biological effect by

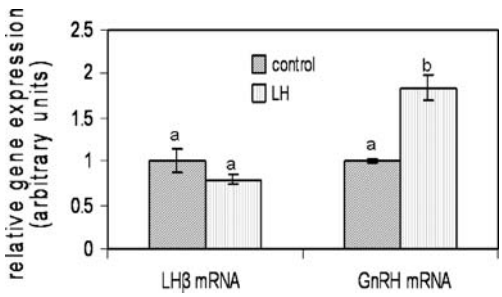

Figure 3 The effect of ovine-LH on the expression of LHβ and GnRH mRNA in granulosa cells from PMSG-primed rats. Granulosa cells isolated from PMSG-primed rats were grown in serum-free medium. On the next day, the medium was replaced with ovine-LH-containing medium (200 ng/ml) for 24 h, and RNA was extracted from the cells. The results are from two experiments, each with triplicates or quadruplicates. *Bars* indicate SEM. *Different letters* indicate statistically significant differences between control and treatment ($P<0.005$)

eliminating the action of the endogenous GnRH. We found (Fig. 4) that in both cultures derived from either estrogen- or PMSG-primed rats, GnRH agonist decreased the expression of LHβ mRNA. On the other hand, the expression of GnRH mRNA was increased by GnRH agonist treatment only in granulosa cells from PMSG-primed rats (Fig. 5). Cetrorelix, a potent GnRH antagonist, had no effect on LHβ or GnRH mRNAs expression in both cell cultures (Figs. 4 and 5).

Discussion

The present study is the first to investigate the regulation of ovarian LHβ mRNA expression in rat granulosa cell cultures. We compared the basal expression of LHβ in granulosa cells from either estrogen- or PMSG-primed rats. The lower basal expression of LHβ mRNA in granulosa cells from PMSG-primed rats, as compared to cells from estrogen-primed rats, results probably from FSH receptor activation by PMSG. This assumption is confirmed by our demonstration of the same effect on LHβ mRNA expression by FSH administrated in vitro to granulosa cells recovered from estrogen-primed rats. It seems that this effect is long lasting, since the level of expression was examined 4 days after PMSG administration (including the 2 days of culture). Analysis of the ovarian LHβ promoter (data not shown) reveals two potential AP-1 sites. Both gonadotropins, FSH and LH, activate the transcription of different combinations of AP-1 binding transcription

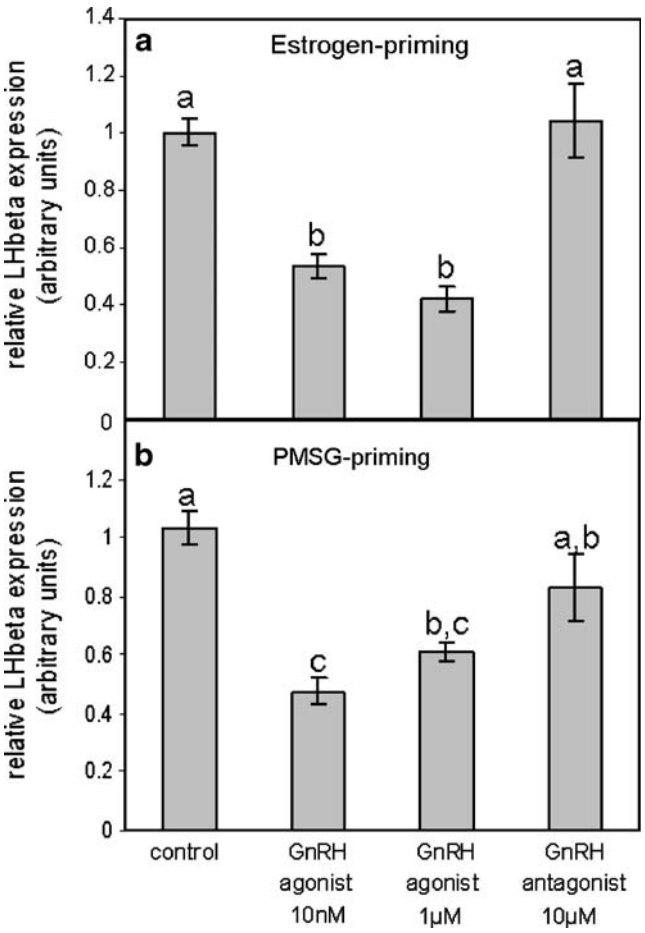

Figure 4 The effect of a GnRH agonist or antagonist on the expression of LHβ mRNA in granulosa cell cultures produced from immature female rats. Granulosa cells isolated either from estrogen- (**a**) or from PMSG- (**b**) primed rats were grown in serum-free medium. On the next day, the cells were treated for 24 h with either D-Trp^{6}GnRH (a GnRH agonist, at 10 nM or 1 μM) or Cetrorelix (a GnRH antagonist, at 10 μM). On the next day, RNA was extracted and relative LHβ mRNA was quantified by real-time PCR. The results are the mean of three experiments, each with triplicates or quadruplicates. *Bars* indicate SEM. *Different letters* indicate statistically significant differences between groups ($P<0.005$)

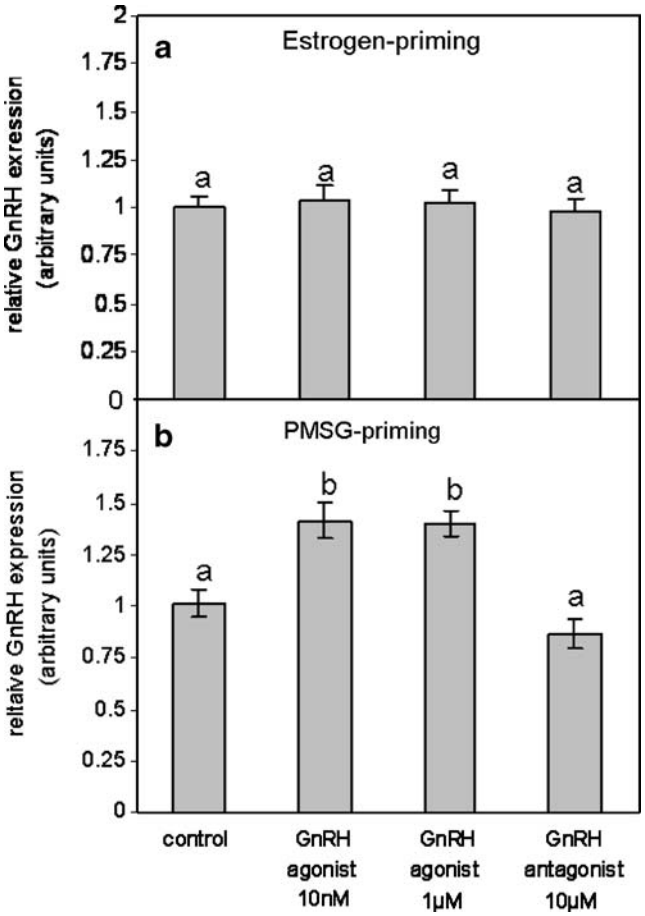

Figure 5 The effect of a GnRH agonist or antagonist on the expression of GnRH mRNA in granulosa cell culture produced from immature female rats. Granulosa cells isolated either from estrogen- (**a**) or from PMSG- (**b**) primed rats were grown in serum-free medium. On the next day, the cells were treated for 24 h with either D-Trp^{6}GnRH (a GnRH agonist, at 10 nM or 1 μM) or Cetrorelix (a GnRH antagonist, at 10 μM). On the next day, RNA was extracted and relative GnRH mRNA was quantified by real-time PCR. The results are the mean of three experiments, each with triplicates or quadruplicates. *Bars* indicate SEM. *Different letters* indicate statistically significant differences between groups ($P<0.005$)

factors, depending on the specific signaling (Sharma and Richards 2000). Thus, it is possible that the down regulation of ovarian LHβ mRNA expression by FSH receptor activation is mediated through one or more of these AP-1 binding sites. Furthermore, these results are in concert with our previous finding that PMSG administration to immature rats reduced the expression of ovarian LHβ mRNA in vivo as compared to untreated rats (Schirman-Hildesheim et al. 2008).

On the other hand, the lower basal expression of GnRH mRNA in granulosa cells from PMSG-primed rats as compared to cells from estrogen-primed rats probably does not represent a direct effect on the FSH receptor, since FSH did not modify the expression of GnRH mRNA in granulosa cells from estrogen-primed rats. Although PMSG might signal through the LH receptor (Vidyashankar and Moudgal 1984), the effect of PMSG was not mediated through the LH receptor, since LH itself had an opposite effect on GnRH mRNA in granulosa cells. Thus, it seems that the effect of PMSG on GnRH mRNA expression in vivo is not a direct effect through the FSH or LH receptor, but that another, yet unknown, factor is responsible for the lower expression of GnRH after PMSG priming. This factor that probably originated in the theca cell layer or in the oocytes may have a significant role in the regulation of ovarian GnRH expression. Indeed, during the estrous cycle, ovarian GnRH mRNA peaks during the proestrous afternoon, but declines at the proestrous evening, after serum LH levels have dramatically increased (Schirman-Hildesheim et al. 2005).

It should also be noted that some differences exist between the regulation of GnRH mRNA in the human, as compared to the rat, granulosa cells. In human granulosa-luteal cells, both gonadotropins, FSH and hCG, reduce the expression of mammalian GnRH mRNA while increasing the expression of the GnRH-II mRNA (Kang et al. 2001), which is not expressed in rodents (Pawson et al. 2003). In contrast, we found that in rat granulosa cells LH increases the expression of GnRH mRNA, while FSH had no effect on GnRH mRNA expression in granulosa cells from estrogen-primed rats. Thus, there are significant differences between human and rat granulosa cells vis-à-vis the regulation of GnRH mRNA. These differences might result as a consequence of the different hormonal regulation of the human menstrual cycle as compared to the rat estrous cycle, or because of the existence of two forms of GnRH peptides in human granulosa cells (Kang et al. 2001) but only one in the rat.

In granulosa cells, like in the pituitary gland, GnRH regulates the expression of LHβ mRNA. But, while GnRH increases LHβ mRNA expression in the pituitary gland (Andrews et al. 1988; Lalloz et al. 1988), rat ovarian LHβ mRNA expression is decreased by GnRH receptor activation. The GnRH agonist, D-Trp^{6}GnRH, reduced the expression of LHβ mRNA in both granulosa cell cultures, derived either from estrogen- or PMSG-primed rats. Since the effects of 10 nM and 1 μM D-Trp^{6}GnRH on the expression of LHβ mRNA were similar, it is clear that the ovarian GnRH receptor is sensitive to low concentrations of GnRH. This virtually may enable regulation of the local LHβ mRNA expression in an autocrine/paracrine fashion. The fact that the GnRH antagonist on its own had no effect on the expression of LHβ mRNA is not clear, as the granulosa cells express GnRH and probably secrete it into the medium. However, Park et al. (2001) found that although a GnRH antagonist had no effect on the pituitary adenylate cyclase-activating polypeptide (PACAP) mRNA expression in granulosa cell culture, it inhibited the LH- and FSH-induced PACAP gene expression. It is possible that some of the actions of GnRH are downstream to those that are elicited by the gonadotropin signaling.

The effect of the GnRH agonist, D-Trp^{6}GnRH, on the expression of GnRH mRNA differed between the two granulosa cell models: in estrogen-primed rats, no change in GnRH mRNA expression was evident, while the expression of GnRH mRNA was increased in granulosa cells from PMSG-primed rats. Accordingly, we found that the hormonal regulation of gene expression in granulosa cell culture depends both on the expression of the GnRH receptor and on the maturational state of the cells. Similarly, the effect of GnRH on the expression of ovarian PACAP mRNA in granulosa cells is also dependent on the maturational state of the granulosa cell culture (Park et al. 2001): a GnRH agonist increased the expression of PACAP mRNA in granulosa cells from PMSG-primed rats, but not from diethylstilbestrol-primed rats. Similarly, the effect of GnRH on the ovary was also found to be dependent on the developmental stage of the follicles. Thus, when GnRH was administered concomitantly with PMSG to hypophysectomized immature rats, GnRH blocked ovulation. However, when GnRH was administered 48 h after PMSG, it induced ovulation (Naor et al. 1983). Similarly, GnRH exerts a stimulatory action on preovulatory follicles by inducing oocyte maturation (Hillensjo and LeMaire 1980) and follicular rupture (Ekholm et al. 1981). However, the effects of GnRH on small follicles are inhibitory in nature, as GnRH analogs were reported to decrease steroidogenesis (Hsueh and Erickson 1979) and ovarian LH receptor binding capacity (Jones and Hsueh 1980). Further investigations are needed to elucidate whether these differences in the functions of GnRH on rat granulosa cells at different follicular states are due to diversities in GnRH receptor signaling at different follicular states or differences in the expression of transcriptional co-activators. Nevertheless, GnRH receptor signaling was found to activate phospholipase-D in granulosa cells independently of the

maturational stage of the cells (Amsterdam et al. 1994; Liscovitch and Amsterdam 1989).

Interestingly, in our studies, treatment of rat granulosa cells with either FSH or GnRH agonist dramatically reduced the expression of LHβ mRNA. This effect was exclusive, as other ovarian and pituitary hormones, such as estrogen, progesterone, and prolactin, did not affect the expression of LHβ mRNA in rat granulosa cells (data not shown). It is possible that LHβ mRNA may be expressed mainly in the immature follicles or before puberty when regulation of ovarian functions by pituitary gonadotropins is only initiated. Although the follicles of LH knockout mice develop up to the pre-antral stage (Kumar 2007; Ma et al. 2004), there are reasons to believe that follicular development before the pre-antral stage may be influenced by gonadotropins. At these stages of follicular development, as early as postnatal day 7 (Siebers et al. 1977; Sokka et al. 1992), theca cells already express an active LH receptor. Indeed, several studies have suggested that the development of pre-antral follicles is regulated by gonadotropins (Funkenstein et al. 1980; McGee et al. 1997).

Our findings suggest the existence of a local, paracrine, GnRH–gonadotropic hormones axis in the gonads. Ovarian-produced GnRH inhibits LHβ mRNA synthesis, while LH that is produced either locally by the ovary or by the pituitary gland induces the expression of GnRH mRNA. Indeed, the minute amounts of the hypothalamic GnRH that are secreted into the pituitary portal system and the rapid degradation of GnRH (Koch et al. 1974; Baram and Koch 1977) suggest that it is the ovarian-produced GnRH that affects granulosa cell gene expression.

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