

Local production of the gonadotropic hormones in the rat ovary

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Abstract

The gonadotropic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are synthesized by and released from the anterior pituitary in response to the hypothalamic gonadotropin-releasing hormone (GnRH) signaling. In the female, LH and FSH affect folliculogenesis, ovarian steroid production, oocyte maturation, ovulation and corpus luteum formation. We have recently studied the expression of GnRH and its receptor in the rat ovary and found organ-specific, estrous cycle-dependant, fluctuations. Subsequently, we wished to determine whether rat ovaries also express gonadotropic hormones. Using RT-PCR, we detected LH β , FSH β and the common α -subunit mRNA's in intact follicles, theca cells, corpora lutea and in meiotically competent and incompetent oocytes. Granulosa cells, however, express mRNA's for LH β and the common α -subunit, but not for FSH β . We cloned and sequenced the ovarian LH β transcript and found it to be longer (2.3 kb) than the one produced by pituitary gonadotropes (0.8 kb), due to a longer 5'-UTR. We studied the regulation of ovarian LH β mRNA in sexually immature female rats administered with pregnant mare serum gonadotropin (PMSG) and in adult cyclic rats. PMSG administration caused a significant decrease in LH β mRNA expression, detected by real-time PCR. Similarly, LH β mRNA levels were lower on estrous morning versus proestrous evening. Interestingly, ovarian content of LH remained unchanged following hypophysectomy, although ovarian weight was immensely reduced. Taken together, it seems probable that ovarian LH is heterologously/homologously regulated by pituitary, and possibly also by local gonadotropins. Thus, these findings may imply the existence of a local GnRH–gonadotropin axis in the mammalian ovary that may be involved in the management of processes that lead to ovulation.

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1. Introduction

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are heterodimeric glycoproteins, produced by the gonadotropes of the anterior pituitary. LH and FSH are composed of a common α -subunit and a specific β -subunit (LH β and FSH β). These gonadotropic hormones are released into the systemic circulation in response to pulsatile secretion of the hypothalamic neuropeptide, gonadotropin-releasing hormone (GnRH). The gonadotropins bind to specific G-protein coupled receptors in the ovary, and affect ovarian folliculogenesis, steroid production, oocyte maturation, ovulation and corpus luteum formation. Although transgenic and knockout animals, with

a dysfunctional gonadotropin or gonadotropin-receptor gene, have been created (Kumar, 2005), the precise effects and signaling pathways activated by LH and FSH in the ovary are not fully understood. Moreover, the regulation of gonadotropin synthesis and release is an intricate interplay of positive and negative feedback loops involving the hypothalamus, pituitary and ovary (Burger et al., 2004).

Interestingly, LH and FSH have been shown to be produced in extra-pituitary tissues, including human, rat and mouse testes (Hovatta et al., 1986; Wahlstrom et al., 1983; Zhang et al., 1995a,b; Berger et al., 1994) as well as in the gilthead sea bream ovary (Wong and Zohar, 2004). Moreover, FSH β and the common α -subunit have been shown to be synthesized by the mouse ovary (Markkula et al., 1996) and LH has been detected in the rat hypothalamus (Emanuele et al., 1981). Similarly, LH receptors are also expressed in extra-gonadal tissues of various mammalian species, such as: the porcine and rabbit uterus (Jensen and Odell, 1988; Ziecik et al., 1986), human adrenal gland and skin (Pabon et al., 1996a,b), and monkey epididymis

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(Zhang et al., 1997). It thus appears that LH might play a local autocrine/paracrine role in extra-gonadal organs. The endogenous gonadal production of LH is intriguing in light of the fact that GnRH and its receptor are also produced locally in the rat and human testis and ovary (Bahk et al., 1995; Choi et al., 2006; Clayton et al., 1992, 1979; Ramakrishnappa et al., 2005; Jones et al., 1980; Oikawa et al., 1990; Dong et al., 1993). The possibility has been raised, that the gonadal LH and GnRH might be functionally related (Wong and Zohar, 2004).

Wong and Zohar (2004) characterized the ovarian transcripts for LH β and FSH β in the gilthead seabream and discovered that, whereas the ovarian FSH β transcript is identical to that in the pituitary, the ovarian LH β transcript is longer due to a \sim 500 bp extension of its 5'-untranslated region (UTR). Furthermore, they demonstrated, using *in vitro* ovarian fragment incubation, that a GnRH agonist enhances LH β and FSH β mRNA expression and increases the secretion of ovarian LH. The latter effect was shown to be blocked by a GnRH antagonist. Like the seabream ovarian LH β transcript, the rat testicular LH β transcript is also longer than the one expressed in the pituitary, due to a longer 5' end (by \sim 1.9 kb). *In situ* hybridization revealed that, in this tissue, LH β is expressed in elongated spermatids (Zhang et al., 1995b).

We have recently studied the expression patterns of GnRH and of GnRH receptor in rat ovaries throughout the estrous cycle, using real-time PCR (Schirman-Hildesheim et al., 2005). We found differential, organ-specific regulation of the expression of GnRH and of its receptor in the ovary as compared to that of the pituitary or hypothalamus. In continuation of this study, and based on previous demonstrations of gonadotropic hormone expression in the seabream ovary and in the rat testis, we set out to determine whether rat ovaries express these hormones. Moreover, we studied the regulation of the LH β transcript during the estrous cycle of adult rats as well as in sexually immature rats that were administered with pregnant mare's serum gonadotropin (PMSG). We report here our novel findings on the expression of gonadotropic hormones in the rat ovary, adding to the growing body of evidence for a local GnRH–gonadotropin axis in the gonads.

2. Materials and methods

2.1. Experimental animals and sample collection

All animals 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., and food and water available *ad libitum*.

2.1.1. Super-ovulation (PMSG-hCG) animal model

Sexually immature 23–24-day-old female Wistar rats were injected s.c. with 10 IU of pregnant mare's serum gonadotropin (PMSG, Chrono-gest Intervet, Boxmeer, The Netherlands) at 10 h to stimulate follicular development to the antral stage. After 48 h, the animals were euthanized and the ovaries were excised. Follicles were isolated for subsequent recovery of granulosa/theca cells, as described below (Sections 2.1.2–2.1.3). Alternatively, 5 IU hCG (Chrono-gest Intervet) were injected i.p. 48 h after PMSG administration and oocytes/corpora

lutea were recovered 24 h later, as described below (Sections 2.1.4–2.1.5). Pituitary glands were dissected out to serve as positive controls of LH measurements.

2.1.2. Isolation of granulosa cells

Forty-eight hours after PMSG administration, follicles were punctured with a 27G needle. Granulosa cells and cumulus–oocyte complexes were gently squeezed out of the follicle. The oocytes were removed and RNA was extracted from the granulosa cells as described below.

2.1.3. Isolation of theca cells

Follicles were punctured; granulosa cells and cumulus–oocyte complexes were gently squeezed out, as described above. Subsequently, the follicles were opened and the follicular wall was gently scraped in order to remove adherent granulosa cells. The remaining tissue thus consisted mainly of theca cells, but may have contained some granulosa and interstitial cells as well. RNA was extracted from theca cells, as described below.

2.1.4. Isolation of oocytes

In a previous study (Goren et al., 1994), we demonstrated that ovaries of 18-day-old Wistar rats contain growing oocytes, which are incompetent to resume meiosis. In contrast, fully grown oocytes that are meiotically competent can be recovered from ovaries of 26-day-old rat, primed with 10 IU PMSG for 48 h. In the present study, we used rats of both age groups (18 and 26 days) as a source of meiotically incompetent and competent oocytes, respectively. For meiotically incompetent oocyte isolation, 18-day-old rats were sacrificed and their ovaries were isolated and transferred into L-15 medium containing 5% FCS. Follicles were punctured and the cumulus–oocyte complexes were recovered and placed into acidic L-15 medium (pH 6.0, Biological Industries) to obtain cumulus-free oocytes. Meiotically competent oocytes, arrested at the second metaphase, were obtained from 26-day-old animals treated according to the superovulation protocol described above. The infundibuli were excised and placed into small Petri dishes containing \sim 1 mg hyaluronidase (Sigma, Rehovot, Israel) in L-15 medium with 5% FCS (Biological Industries). Infundibuli were torn apart and milked to dislodge ovulated cumulus–oocyte complexes. The added hyaluronidase dispersed the cumulus mass to obtain cumulus-free oocytes. RNA was extracted from oocytes as described below.

2.1.5. Isolation of corpora lutea

Corpora lutea were obtained from 26-day-old animals treated according to the superovulation protocol described above. The animals were sacrificed 24 h after hCG treatment and their ovaries were removed and placed in L-15 medium with 5% FCS. Then, corpora lutea were isolated using tweezers and collected into a tube. RNA was extracted from corpora lutea, as described below (Section 3.3).

2.1.6. Effect of PMSG administration on LH β mRNA expression

Sexually immature 24 day-old female Wistar rats were injected s.c. with 10 IU of PMSG at 10:00 h and sacrificed 12 h later ($n=4$). Untreated 24-day-old rats were sacrificed at 10:00 h ($n=4$) or at 22:00 h ($n=4$), to control for time-of-day effects. After sacrifice, ovaries were immediately removed and placed in 10 volumes of RNA Later (Ambion, Austin, TX, USA) until subsequent RNA extraction.

2.1.7. LH β mRNA expression in adult cyclic rats

Estrus cycles of 7–9 weeks old female Wistar rats were monitored via vaginal smears and only rats showing at least 3 consecutive 4-day cycles were used. Animals were sacrificed on proestrus at 18:30 h ($n=10$) or on estrus at 09:00 h ($n=7$). In our colony, the preovulatory gonadotropin surge occurs at around 18:30 h on proestrus (Schirman-Hildesheim et al., 2005). Ovaries were immediately removed and placed in 10 volumes of RNA Later (Ambion) until subsequent RNA extraction.

2.2. PCR primers

Primers for all genes were designed on two different exons so as to span one intronic sequence. The sequences of the PCR primers used to detect LH β (acces-

sion NM_012858) expression are: *forward primer* 5'-ctgtctagcatggtcag-3' and *reverse primer* 5'-agttagtggtggcatcag-3'. The forward primer spans the boundary between exons 2 and 3, whereas the reverse primer resides on exon 3 such as to amplify nucleotides 181 (exon 2) to 452 (exon 3) of the LH β mRNA, producing a 271 bp product. The sequences of the PCR primers used to detect FSH β expression are: *forward primer* 5'-aggaagagtgccttctgc-3' and *reverse primer* 5'-gctgctacatcacactgc-3' (Fujii et al., 2002). These primers amplify nucleotides 98 (exon 2) to 330 (exon 3) of the FSH β mRNA (accession NM_001007597), yielding a 233 bp product. The sequences of the PCR primers used to detect α -subunit gene expression are: *forward primer* 5'-ctatcagtgtatggctgttc-3' and *reverse primer* 5'-cttggtagtaacaagtc-3' (Fujii et al., 2002). These primers amplify nucleotides 697 (exon 3) to 895 (exon 4) of the α -subunit gene (accession AH003617), producing a 199 bp mRNA product.

The primer sequences for the reference genes, their PCR product sizes, and annealing temperatures were described previously (Schirman-Hildesheim et al., 2005). To verify the identity of PCR products obtained using each primer combination, each product was loaded onto a 1.8% ethidium bromide-stained agarose gel and the resulting band was purified and sequenced (DBS-QBI Bioresearch Services, Rehovot, Israel).

2.3. RT-PCR detection of the common α -subunit, LH β and FSH β

Total RNA was extracted from granulosa and theca cells, corpora lutea, meiotically competent and incompetent oocytes using Tri-Reagent (Sigma), according to the manufacturer's recommendations. The RNA pellet obtained from oocytes was dissolved in 9 μ l sterile double-distilled water (ddW) and 20 μ l for granulosa or theca. Granulosa and theca RNA concentrations were quantified using a NanoDrop machine (NanoDrop Technologies, Wilmington, DE, USA) and their RNA purity was assessed on the same machine using the 260:280 and 260:230 nM ratios.

Reverse transcription (RT) was performed using the Muloney murine leukemia virus reverse transcriptase—Ribonuclease H⁺ enzyme (M-MLV RNase H⁺, Promega, Madison, WI, USA). Each reaction contained: 8 μ l oocytes total RNA or 1.5 μ g granulosa or theca total RNA, 4 μ l of the M-MLV 5 \times buffer (Promega), 10 mM deoxynucleotide triphosphates (dNTP, MBI Fermentas, St. Leon-Rot, Germany), 0.5 μ g Oligo (dT)_{12–18} (Promega), 40 units of the RNase inhibitor RNasin (Promega), 200 units of the M-MLV RT-RNase H⁺ enzyme and 3 μ l sterile ddW. This mixture was incubated at 37 °C for 2 h.

Subsequently, a 2 μ l aliquot of the oocyte, granulosa or theca cDNA were used in a PCR reaction containing: 10 μ l of the PCR master mix "Reddymix" (ABgene, Surrey, UK), 250 nM of each the forward and reverse LH β or FSH β primer and UltraPure PCR-grade water (Fisher Biotec, Subiaco, WA, Australia) to a final volume of 20 μ l. PCR was carried out in a Palm-Cycler (Corbett Research, Sydney, Australia), with the following protocol: 3 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 30 s at 60 °C (for LH β and α -subunit amplification) or 55 °C (for FSH β amplification) and 15 s at 72 °C, with an additional 7 min at 72 °C at the end of the cycling protocol. An additional PCR reaction, which contained nuclease-free water instead of cDNA served as a negative control. PCR products were then loaded onto a 1.8% ethidium bromide-stained agarose gel and photographed under the UV illumination of a MultiImage Light Cabinet by the Chem-Imager 4000 software (Alpha Innotech Corporation).

2.4. Measurement of LH β expression in ovaries of PMSG-treated immature rats and adult cyclic rats using real-time RT-PCR

RNA extraction, reverse transcription, real-time PCR and calculation of relative mRNA abundance, were carried out exactly as described previously (Schirman-Hildesheim et al., 2005). For normalization of gene expression, a panel of four candidate reference genes (*HPRT*, *Cyclophilin*, *RPL19* and β -actin) were tested in all experimental samples in order to identify the most stably expressed genes in all tested times. Primer sequences and reaction conditions for all tested reference genes, as well as the criteria used to select the most stable reference genes, have been described previously (Schirman-Hildesheim et al., 2005).

2.5. Characterization of the rat ovarian LH β transcript

The sequence of the ovarian LH β transcript was determined as follows: ovarian RNA was reverse transcribed using SuperScript II RNase H⁻ Reverse Transcriptase kit (Invitrogen Life Technologies, Carlsbad, CA, USA) and oligo-dT (Amersham Biosciences, Piscataway, NJ, USA), according to the manufacturer's instructions. PCR was performed using primers designed according to the testis LH β transcript (accession U25653). The sense primer: 5'-CCAAGCTTGAGGGTGAGGACCATGGCA-3' included a 5'-digestion site for Hind-III, and the antisense primer: 5'-CCGCTCGAGAAGCCTTTATTGGGAGGGAT-3' included a 5'-digestion site for Xho-I. The resulting PCR fragment was cloned into a pcDNA3 plasmid (Invitrogen Life Technologies). The plasmid was sequenced using the following primers: a T7-primer, the antisense primer used for cloning, and two additional primers: 5'-AGCCAGTGTCTATTCTATCC-3' and 5'-GAAATCGGAGATTGCCAGA-3'.

2.6. LH content quantification using radioimmunoassay

Pituitary glands, oocytes or follicles were collected as described above and homogenized in a glass-Teflon homogenizer. They were then subjected to 10 freeze–thaw cycles in the hypotonic Tris-buffered solution (5 mM). Homogenates were centrifuged at 14,000 rpm for 10 min in an Eppendorph centrifuge cooled to 4 °C. The resulting supernatant was taken for quantification of LH content by RIA.

The exact procedure employed for the quantification of LH using RIA was described previously (Yahalom et al., 2000). LH content was determined in three different sample dilutions using the kit for rat LH, provided by the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD) Rat Pituitary Program. LH levels were computed in terms of the RP-3 reference preparation. The intra-assay coefficient of variation for LH RIA was 2%, and the assay sensitivity was 20 pg/tube.

2.7. Statistical analyses

Statistical analyses were performed using JMP IN Statistical Discovery Software, Version 5.1 (SAS Institute Inc., Cary, NC, USA). Statistical evaluation of differences between time/treatment groups was performed using pair-wise comparisons of means using the Student's *t*-test (LSD) at confidence level of 95%. Further details were described previously (Schirman-Hildesheim et al., 2005, 2006).

3. Results

3.1. LH β , FSH β and common α -subunit mRNA expression in ovarian cells

Using RT-PCR, we detected LH β mRNA (Fig. 1A), FSH β mRNA (Fig. 1B), as well as the common α -subunit mRNA (Fig. 1C) in theca cells, corpora lutea, meiotically competent and incompetent oocytes, and in the pituitary gland of the rat. In granulosa cells we detected only LH β and α -subunit transcripts but not the FSH β mRNA. We also found that in several non-reproductive tissues, such as muscle and liver, only the α -subunit is expressed (data not shown). The gonadotropic hormone subunit transcripts were detected using primers that span an intronic sequence. To ascertain the identity of the obtained PCR products, we sequenced the amplicons and compared them to the known gonadotropic hormone subunit transcripts expressed in the anterior pituitary. A 100% identity between the ovarian and pituitary sequences was obtained, confirming that we have indeed amplified the gonadotropic hormones' α - and β -subunit mRNA's.

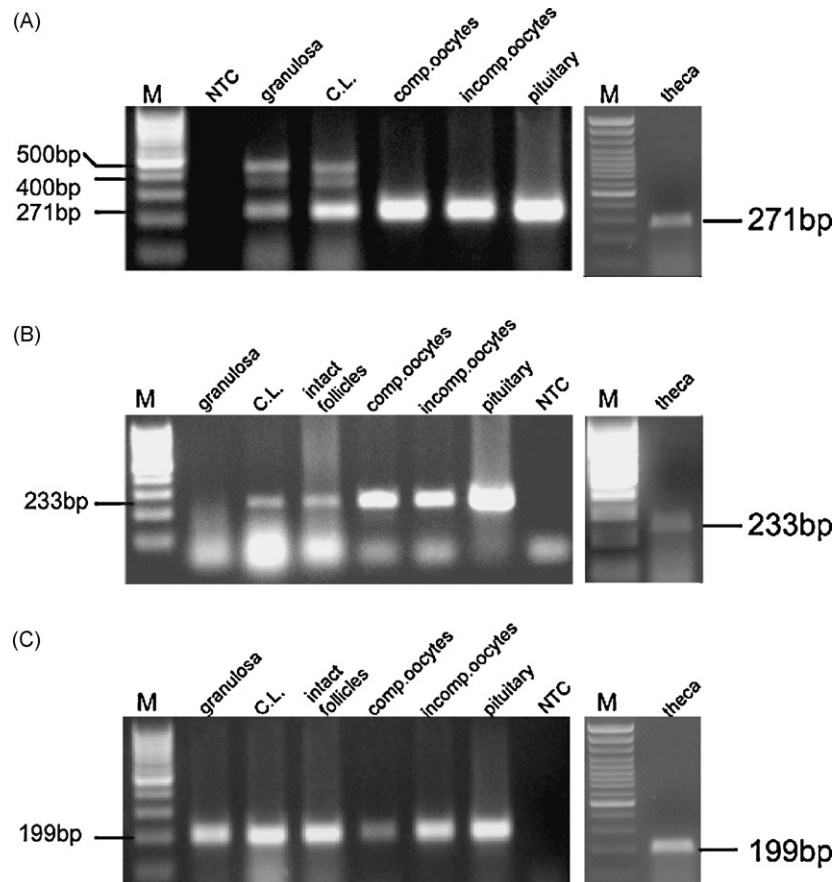


Fig. 1. (A) LH β , (B) FSH β and (C) α -subunit mRNA expression in rat ovarian follicle cells. Total RNA's were extracted and reverse-transcribed (RT) as described in Section 2. The resulting cDNA's (labeled on top of each gel image) were subjected to PCR using (A) LH β -specific primers, spanning nucleotides 181 (exon 2) to 452 (exon 3) of the LH β gene (accession NM_012858), producing a 271 bp mRNA product. Note that 2 additional LH β variants (400, 500 bp) are evident in granulosa and CL cells but not in theca and oocytes. (B) FSH β -specific primers (Fujii et al., 2002), yielding a 233 bp amplicon spanning nucleotides 98 (exon 2) to 330 (exon 3) of the FSH β gene (accession NM_001007597). Note that FSH β is not detected in granulosa cells. (C) α -subunit-specific primers (Zhang et al., 1995a), spanning nucleotides 697 (exon 3) to 895 (exon 4) of the α -subunit gene (accession AH003617), producing a 199 bp mRNA product. A no-template control (NTC) reaction included water instead of cDNA in each assay. M, DNA ladder; C.L., corpus luteum; comp./incomp. oocytes, meiotically competent or incompetent oocytes.

When cDNA's derived either from granulosa cells or from the corpus luteum were used for the amplification of LH β mRNA, we observed two larger PCR-products (400 and 500 bp; see Fig. 1A), in addition to the expected 271 bp band. These additional bands were sequenced and found to contain intronic sequences from intron B (between exons 2 and 3) of the LH β gene. Since RNA samples were treated with DNase to avoid genomic DNA contamination, we believe that these larger PCR products might represent alternative splice forms of the LH β transcript in granulosa cells and corpora lutea.

3.2. Characterization of the ovarian LH β transcript

We cloned and sequenced the transcript of the ovarian LH β gene using primers that were designed according to the testis LH β transcript (accession U25653). We found that the ovarian LH β transcript (2.3 kb) is identical to that produced in the testes and is thus longer than the pituitary transcript (0.8 kb) due to an extension of its 5'-UTR.

3.3. Quantitation of the LH content in oocytes and in ovarian follicles of intact and hypophysectomized rats

We measured the content of LH in oocytes and follicles using RIA, and compared the obtained values to those found in the pituitary gland. We found approximately 40 fg LH per each oocyte (2200 oocytes were pooled together) and 5.5 pg LH per one intact follicle (80 follicles were pooled together). Each pituitary gland of the 26-day-old rats contained 4–6 μ g LH. To ensure that the LH that was found in the ovary is not pituitary-derived LH that is bound to ovarian receptors, we used hypophysectomized (HPX) rats on the 12th day after surgery. We found that in the HPX rats the LH content was similar to that of normal rats of the same age (about 0.2 ng LH per ovary, average of 7 ovaries from 5 different rats). Moreover, taking into account that the ovaries of the HPX rats weighed 60–70% less than those of intact rats, it seems that the production of LH in the HPX ovaries is much higher than in the normal ovary.

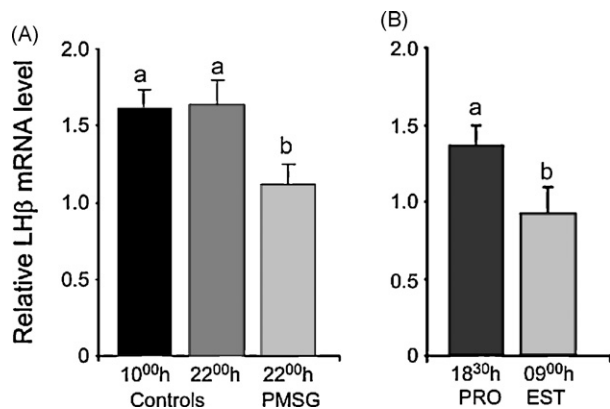


Fig. 2. LH β expression in the ovary following PMSG administration (A) and in adult cyclic rats (B). (A) 24-day-old animals were administered with PMSG at 10 h and sacrificed at 22 h. Untreated age-matched control animals were sacrificed at 10 and 22 h ($n=4$). LH β expression in the ovary is significantly lower at 22 h in PMSG-administered rats vs. either group of control animals ($p<0.05$). (B) 7–9 week old rats were sacrificed at 18:30 h on proestrus ($n=10$) and at 09:00 on estrus ($n=7$). The ovarian LH β expression is significantly lower on estrous morning vs. proestrous evening ($p<0.05$). Values are the mean relative ovarian LH β mRNA levels (arbitrary values) \pm S.E.M., calculated as described in Section 2. Different letters indicate statistically significant differences between groups (Student's t -test, $p<0.05$). PRO, proestrus; EST, estrus.

3.4. Effect of PMSG on LH β mRNA expression in the ovary

Using computational methods, which we have previously described, to select for the most appropriate reference genes among a set of samples (Schirman-Hildesheim et al., 2005), we found that β -actin and RPL19 mRNA levels are unaffected by PMSG administration. These two genes were therefore used as normalizers of LH β expression.

Using quantitative PCR, we found that 12 h after PMSG administration to sexually immature female rats, LH β expression in the ovary was reduced by 33% (Fig. 2A, $p<0.05$) as compared to ovaries taken from untreated rats that were sacrificed either at the time of PMSG administration or 12 h later (Fig. 2A). These reduced levels of LH β mRNA following PMSG administration persisted for up to 48 h ($p<0.05$, data not shown). Similarly, using quantitative PCR, we found that in adult cyclic rats, LH β mRNA levels declined by about 33% between the evening of proestrus (time of the endogenous LH surge) and the morning of estrus ($p<0.05$; Fig. 2B).

4. Discussion

The present study demonstrates for the first time that gonadotropic hormones are synthesized in the mammalian ovarian follicle and in oocytes. Assuming that these hormones act locally in the ovary, in an autocrine/paracrine fashion, the small quantity of LH peptide that we detected in ovarian follicles and oocytes, as compared to the pituitary, is not surprising. Moreover, whereas the pituitary gland (i) synthesizes, (ii) stores and (iii) releases hormones, and each of these steps could be regulated separately, the ovary might not store the gonadotropic hormones, which it produces. Indeed, hormones produced by

the placenta (i.e. human placenta lactogen, human chorionic gonadotropin) are secreted and are not stored in the placenta (Handwerger, 1991; Walker et al., 1991). Moreover, although LH and FSH are synthesized in the same pituitary gonadotropes, their storage and secretion patterns are different. Thus, the majority of the newly synthesized FSH is released constitutively (Muyan et al., 1994). Indeed, it is probable that the ovarian gonadotropes are produced “on-demand”, thus the regulation of their expression may be the main mechanism that controls their function.

Wahlstrom et al. (1983) were the first to report the finding of LH, FSH in human and rat testis. Using immunohistochemistry, they detected LH positive cells in human and rat Leydig cells whereas FSH was localized to Sertoli cells. Ten years later, Berger et al. (1994) reported the expression of both hCG β and LH β in cytosolic extracts of human testis. Shortly afterwards, the group of Huhtaniemi found that LH β and the common- α subunit are expressed in the rat testis, and that this expression is localized to elongated spermatids (Zhang et al., 1995a). They further found that rat testis express three LH β transcripts, of different length (Zhang et al., 1995b). They characterized these mRNA species, which result from alternative splicing, and reported that the main species (2.7 kb) is longer than the pituitary transcript (0.8 kb) due to an extension at the 5'-end. Subsequently, Wong and Zohar (2004) reported on gonadotropic hormone production in the oocytes of the gilthead sea bream. In this fish, the ovarian LH β transcript (1.1 kb) is longer than the pituitary mRNA (0.6 kb), due to an extension at the 5'-UTR. Similar to the situation described for both the ovary of the gilthead sea bream (Wong and Zohar, 2004) and the rat testis (Zhang et al., 1995a), we have found that the ovarian LH β transcript is longer than the known pituitary transcript (0.8 kb), due to a longer 5'-UTR. The functional significance of this finding is yet to be unveiled. It is possible however, that the longer 5'-UTR in the ovarian transcript serves an organ-specific regulatory purpose, at the post-transcriptional level.

In the present study, we measured the effect of PMSG administration on LH β mRNA abundance and found that it causes a significant reduction in the endogenous level of ovarian LH β mRNA (Fig. 2A). This might indicate that the ovarian LH is homologously regulated and/or that it is regulated by FSH. Though the physiological significance of this finding is to be explored, it is possible that, in the adult cyclic rat, the endogenous ovarian LH is down-regulated by the preovulatory gonadotropin surge. Indeed, we did observe a decrease in the abundance of LH β mRNA in the morning of estrus, as compared to the approximate time of the LH surge on proestrus (Fig. 2B). This could provide for a feedback mechanism between the pituitary and the local ovarian gonadotropic hormones.

Various studies reported multiple functions of GnRH in the rat gonads (Hsueh and Schaeffer, 1985). However, the observation that GnRH induces ovulation in hypophysectomized rats (Corbin and Bex, 1981), has triggered the establishment of a new concept in reproductive endocrinology, suggesting that GnRH acts directly on the ovary without the mediation of the pituitary hormones, and is capable of inducing various responses, such as

ovulation or changes in steroid hormones metabolism. Our findings, together with the publications discussed above, suggest the existence of a local, paracrine, GnRH–gonadotropic hormones axis in the gonads. Although the reproductive system, by and large, is regulated by the hypothalamus–pituitary–gonadal axis, it is possible that ovarian functions may possess a greater degree of autonomy than it is currently conceived. Indeed, the ovary produces the entire set of hormones and their receptors that are currently known to regulate reproduction, including: GnRH and its receptor, the gonadotropic hormones and their receptors, activin, inhibin, follistatin, prolactin and their receptors (Clayton et al., 1992; Jones et al., 1980; Oikawa et al., 1990; Frasar and Gibori, 2003; Lewis et al., 2000; Whitelaw et al., 1995). Thus, the ovary is capable of managing a local, restricted, control station under the supervision of the central feedback mechanism. There is no doubt that the regulation of the LH surge, which leads to ovulation, is under hypothalamic control. However, it is possible that several tasks such as the fine control of follicular development, the initial stages of ovum maturation, steroidogenesis, etc., may be regulated by intra-ovarian processes that “prime” the developing follicle in anticipation for the preovulatory surge of the pituitary gonadotropins. The latter might then negatively feed-back upon the ovarian gonadotropins to signal the end of their mission.

Hence, it would be important to elucidate the physiological roles of the local ovarian GnRH and gonadotropins in the management of processes that lead to ovulation. Clearly, the creation of genetically modified animals with conditional or inborn ovary-specific knockout of the genes encoding LH, FSH, and both, would be instrumental in further elucidating the role of these locally produced hormones in ovarian function and to distinguish their actions from those of their pituitary homologs.

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