Fetal expression of GnRH and GnRH receptor genes in rat testis and ovary

M-C Botte1, A-M Chamagne2, M-C Carre1, R Counis1 and M-L Kottler1,3

1Laboratoire d’Endocrinologie Cellulaire et Moléculaire de la Reproduction, Université P&M Curie, CNRS URA 1449, 75005 Paris, France, 2CNRS URA 2115, CHU Pitié-Salpêtrière, 75013 Paris, France and 3Laboratoire de Biochimie Médicale, CHU Pitié-Salpêtrière, 75013 Paris, France

Requests for offprints should be addressed to M-C Botte, Laboratoire d’Endocrinologie Cellulaire et Moléculaire de la Reproduction, Université P&M Curie, CNRS URA 1449, case 244, 4 place Jussieu, 75252 Paris Cedex 05, France

Abstract

The identification of gonadal gonadotropin-releasing hormone receptors (GnRH-R) and evidence of direct inhibitory effects of GnRH agonists upon steroidogenesis in adult rat gonads, lend credence to a putative intragonadal role of a locally secreted GnRH or GnRH-like peptide. Using reverse transcription-polymerase chain reaction followed by Southern blot hybridization and sequencing, we identified, both in the ovary and in the testis of fetal and adult rats, a fully processed GnRH messenger RNA (mRNA), the sequence of which, in adult testis, was identical to that found in the hypothalamus. We also detected in the testis, but not in the ovary, a transcript containing the first intron.

The ontogeny of GnRH and GnRH-R gene expression was studied in rat gonads from 14·5 to 21·5 days post-coitum (dpc), using dot blot hybridization of total RNA. During this period, the levels of cyclophilin mRNA normalized to total RNA remained unchanged. Thus, we used cyclophilin as an internal standard. GnRH mRNA was detected in the ovary at 18·5 dpc, four days later than in the testis, and similar levels were found in both sexes at birth. GnRH-R mRNA was present at 14·5 dpc in the testis and at 15·5 dpc in the ovary, with the levels at 21·5 dpc being 2·4 times higher in the testis than in the ovary. GnRH and GnRH-R mRNA levels increased in both sexes in late fetal development, but this increase appeared two days sooner in the ovary compared with the testis, thus supporting the hypothesis that expression of the GnRH and GnRH-R genes is regulated in a sex-dependent manner during fetal development. In all cases, expression of GnRH and GnRH-R preceded gonadotropin receptors in the gonads and initiation of gonadotropin secretion by the pituitary.

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Introduction

Gonadotropin-releasing hormone (GnRH) is a hypothalamic decapeptide that plays a key role in the neurohormonal control of reproduction by stimulating the release of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), via specific membrane receptors (GnRH-R) of the gonadotrope cells. These gonadotropins promote, in turn, the development of gonadal functions such as gametogenesis and steroidogenesis.

There is strong evidence for the presence of specific GnRH or GnRH-like binding sites on the gonads of the adult rat (Hsueh & Jones 1981). Radioligand binding assays first demonstrated their existence on Leydig cells (Clayton et al. 1980, Lefebvre et al. 1980, Sharpe & Fraser 1980a) and on granulosa and luteal cells (Clayton et al. 1979, Harwood et al. 1980a,b, Reeves et al. 1980). We have shown that the primary structure of this gonadal GnRH-R is identical to the pituitary one (Moumni et al. 1994). Since the hypothalamic GnRH cannot be the ligand of this gonadal GnRH-R, because of its low concentration in the general circulation (Nett et al. 1974) and of its short half-life (Eskay et al. 1977, Hsueh & Jones 1981), the existence of an endogenous GnRH-like peptide was suspected. It was demonstrated in Sertoli cells by competition studies and immunochemistry (Sharpe & Fraser 1980b, Paull et al. 1981, Bhasin et al. 1983), while a peptide with binding activity similar to GnRH was described in granulosa cells (Aten et al. 1986). However, to date the exact nature of this peptide and its role in gonadal paracrine and autocrine regulation remain uncertain.

In vivo studies in male or female adult hypophysectomized rats have shown that exogenous GnRH or GnRH agonists can both stimulate and inhibit gonadal functions, especially steroidogenesis (Hsueh & Jones 1981). In males, for example, when a GnRH agonist is administered at a...
Materials and Methods

Animals and tissues

Female and male rats (Sprague–Dawley, Centre d’Elevage Janvier, Le Genest, France) were housed under controlled photoperiods (12 h light:12 h darkness), with water and food available ad libitum. Males were caged with the females for one night. Since the estimated time of ovulation was 0200 h, the day following an overnight mating was counted as day 0-5. Pregnant rats were killed at the same hour 14·5 to 21·5 days post-cotum (dpc), and fetuses were removed. The sex of the fetuses was determined by morphological differences and the gonads were dissected together with adjacent mesonephros for 14·5 and 15·5 dpc fetuses, or without mesonephros for 16·5 to 21·5 dpc fetuses. In addition, the stomach and the limb buds were taken as negative controls for dot blot hybridization. The ovaries were not taken from 14·5 dpc fetuses because they were difficult to excise without contamination by other tissues. After a brief rinse in normal saline solution, the tissues were rapidly frozen in liquid nitrogen and stored at −80°C until use.

Postnatal testes were also removed from 4 day post-partum (dpp), 20 dpp and adult (5 months) rats, frozen in liquid nitrogen and stored at −80°C.

RNA extraction

Gonads from the same stage of development were pooled to obtain at least 60 µg RNA for both dot blot and RT–PCR studies. Total RNA was extracted using the Tri-Insta-Pure procedure (Eurogentec, Seraing, Belgium) (Chomczynski & Sacchi 1987).

Reverse transcription and polymerase chain reaction

Total RNA was reverse transcribed to complementary DNA (cDNA) using oligo-dT primers and Superscript II reverse transcriptase (Gibco-BRL, Paris, France), and PCRs were performed with 1 U Taq DNA polymerase (Eurobio, Les Ulis, France) under conditions previously described (Moumni et al. 1994). The sets of forward (F) and reverse (R) primers (Genset, Paris, France) used are presented in Fig. 1. F1-R1 was specific for the rat hypothalamic GnRH cDNA (Adelman et al. 1986) (Fig. 1A), and F2-R2 (Moumni et al. 1994) was specific for the rat pituitary GnRH-R cDNA (Fig. 1B). Amplifications were carried out in a programmable thermal cycler (MJ Research Inc., Watertown, MA, USA) using two different programs of 35 cycles, optimized for GnRH cDNA (50 s at 95°C, 50 s at 50°C, 40 s at 73°C), and GnRH-R cDNA (1 min at 95°C, 1 min at 55°C, 1 min at 72°C). Both programs were terminated by an extension of 10 min at 72°C. For each run of RT–PCR, two negative controls were systematically added, in which water replaced RNA during the RT preparation, or cDNA during the PCR preparation.

Probes and labeling

cDNA probes were used for dot blot hybridization. The GnRH-R probe was described by Moumni et al. (1994) and the cyclophilin probe was a gift from Dr J Douglass (Danielson et al. 1988). The GnRH probe was a 344 bp cDNA encompassing the entire coding sequence of the GnRH precursor and 68 nucleotides of its 3’-untranslated region (Adelman et al. 1986). Probes were labeled by random priming with [α-32P]dCTP (specific activity 3000Ci/mmol) (NEN-Dupont de Nemours, Les Ulis, France) using an Amersham Megaprime labeling kit (Amersham, Arlington Heights, IL, USA).

Oligonucleotide probes (Genset) were used for Southern blot hybridization. The sequences and positions of the probes were selected according to the rat GnRH gene sequence (Bond et al. 1989) and are indicated on Fig. 1A: the exonic probe E was selected in the second exon, and the intronic probes i1, i2, i3 were selected in the first, second and third intron respectively. A probe J was...
selected in the first exon of the GnRH-R gene (Reinhart et al. 1997) as indicated in Fig. 1B. These probes were labeled with $[^\gamma\text{-}32\text{P}]$ATP (specific activity 3000 Ci/mmol) and T4 polynucleotide kinase (Promega Biotec, Charbonnières, France).

**Southern analysis of PCR products**

Amplified DNA was electrophoresed on 1·2% agarose gels in Tris–borate–EDTA buffer together with a DNA marker (PhiX-174/HaeIII digested), in the presence of ethidium bromide. DNA was then alkaline-transferred onto nylon filters (Hybond-N, Amersham) and the filters were hybridized (Sambrook et al. 1989). Labeled hybrids were revealed by autoradiography using Kodak AR X-OMAT films (Eastman Kodak, Rochester, NY, USA).

**Cloning and sequencing of PCR products**

PCR products were purified from agarose using a Qiaex DNA Gel Extraction kit (Qiagen, Courtabœuf, France) and cloned into pUC18 vectors (Surclone Ligation kit, Pharmacia Biotech, Courtabœuf, France) as previously described (Moumni et al. 1994). Recombinant plasmid DNA was sequenced bidirectionally using the Sequenase sequencing system version 2·0 (US Biochemicals/....
Amersham), and M13 universal and reverse primers. Sequence analysis was performed using DNA Strider (Marck 1988).

**Dot blot hybridization of fetal tissues RNA**

Total RNA from pools of fetal organs of the same stage of development was formaldehyde denatured and diluted aliquots were spotted onto two Protran filters (Schleicher and Schuell, Ecquevilly, France) using a Bio-Rad apparatus (Bio-Rad Laboratories, Richmond, CA, USA). Filter 1 (µg total RNA, 5, 10 and 15 µg aliquots) was hybridized with the [32P]-labeled GnRH cDNA. Filter 2 (2·5, 5 and 10 µg aliquots) was hybridized with the [32P]-labeled GnRH-R cDNA. Then both filters were stripped and rehybridized with freshly labeled GnRH-R (filter 1) and GnRH (filter 2) cDNA probes. Finally, both filters were stripped and rehybridized with the [32P]-labeled cyclophilin probe. Stripping efficiency was systematically controlled by autoradiography. Hybridization and washings were performed using standard methods as previously described (Garrel et al. 1993). Radioactivity on the filters was revealed by autoradiography (Kodak X-Omat AR) and quantified by densitometry of the entire surface of the spot using an Autorad 210 device (IMSTAR, Paris, France).

**Data analysis**

The densitometric data obtained for cyclophilin mRNA were normalized against the total RNA loaded. The results were presented after fitting to the most appropriate model of regression. The significance of the determination coefficient (R²) was assessed using Fisher–Snedecor test, and that of the slope (a) using Student’s t-test. A value of P ≤ 0·05 was considered significant. The data for GnRH and GnRH-R mRNA were corrected for variability in loading by expressing the values in arbitrary units as a ratio to cyclophilin.

**Results**

**Gonads collection and RNA extraction**

For the study, a total of 481 fetuses from 42 litters was used. The precise number of fetal gonads at each stage of development and the estimated amount of total RNA extracted per gonad, are indicated in Table 1. During the last three days of gestation the amount of total RNA extracted per testis was higher than that extracted per ovary (1·9 to 5·9 µg vs 1·5 to 2·7 µg respectively), whereas it was equal in both sexes until 18·5 dpc.

**PCR analysis of GnRH mRNA in adult, immature and fetal gonads**

After reverse transcription of total RNA extracted from 14·5 to 21·5 dpc testes, from 15·5 to 21·5 dpc ovaries, and from 4 dpp, 20 dpp and adult testes, parallel amplifications of cDNA were performed using F1–R1. Gel electrophoresis of the products are shown in Fig. 2. The lack of signal in the control RT and PCR reactions omitting template, demonstrated the absence of DNA contaminants and the specificity of the PCR reactions. Two major fragments with sizes of approximately 1280 and 480 bp, were found for 4 dpp (P4), 20 dpp (P20) and adult (Ad) testes (panel A–I). Both hybridized to the exonic [32P]-labeled probe E (panel A–II). The smallest form was present in larger amounts and its size was consistent with the expected size of 477 bp deduced from the hypothalamic sequence (Adelman et al. 1986). To characterize these fragments further, we determined the nucleotide sequence of the

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### Table 1 Characteristics of the fetal gonad collection

<table>
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<th>Stage of development (dpc)</th>
<th>Number of litters/total number of fetuses</th>
<th>Number of testes</th>
<th>Number of ovaries</th>
<th>Total RNA extracted/testis (µg)</th>
<th>Total RNA extracted/ovary (µg)</th>
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<td>—</td>
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477 bp PCR product obtained from adult testis and investigated the presence of intronic sequences in the 1280 bp amplified fragments. We found a complete homology between the adult 477 bp GnRH cDNA sequence in the testis and that in the hypothalamus (Adelman et al. 1986). The hybridization of the 1280 bp fragment with [32P]-labeled intronic probes revealed that i1 (panel A-III), but not i2 or i3 (data not shown), recognized this PCR product, the size of which was consistent with the conservation of the first intronic region (800 bp) in addition to the 477 bp amplified fragment (Bond et al. 1989).

In fetal testes (panel A-I, lanes 14·5 to 21·5) as well as in fetal ovaries (panel B-I, lanes 15·5 to 21·5), the gel analysis revealed a major fragment having the expected size of 477 bp. In addition, the 1280 bp fragment was visualized as a very weak signal in all the fetal testes (panel A-I, lanes 14·5 to 21·5) but not in the fetal ovaries (panel B-I, lanes 15·5 to 21·5). Its identity was confirmed after hybridization with E and i1 as described above (testes: panels A-II and A-III; ovaries: panels B-II and B-III). All signals were readily apparent after a longer time of exposure (data not shown).

Identification of GnRH-R cDNA in fetal gonads

Parallel amplifications of cDNA obtained from the same fetal gonads were performed using F2-R2. Gel electrophoresis of the products is represented in Fig. 3. A single fragment of about 1040 bp consistent with the expected size deduced from the adult gonadal cDNA sequence (Moumni et al. 1994) was visualized by ethidium bromide, for all fetal testes (panel I-A, lanes 14·5 to 21·5) and ovaries (panel I-B, 15·5 to 21·5), but not for negative controls (lanes RT and PCR). The identity of this fragment was confirmed after hybridization to the internal [32P]-labeled probe J (panel II-A: testes, panel II-B: ovaries).

Evolution of cyclophilin mRNA levels in fetal gonads

The amount of cyclophilin mRNA present in the total RNA extracted from fetal gonads is represented as a function of fetal stage in Fig. 4. Since all filters were processed together for hybridization with the cyclophilin probe and exposure to X-ray film, the results were expressed as arbitrary units. The cyclophilin mRNA levels are in the range of 0·04 arbitrary units for fetal testes, and 0·025 arbitrary units for fetal ovaries. In both cases, values fitted a linear curve of regression with a slope and a correlation coefficient close to zero (P>0·05).

Evolution of GnRH and GnRH-R mRNA in gonads during fetal development

Densitometric data obtained for GnRH and GnRH-R mRNA were analyzed after standardization with cyclophilin, and expressed as arbitrary units. Since RNA extracted from fetal testes and ovaries were loaded onto the same filter and hybridized together with the same labeled probes, GnRH and GnRH-R gene expression were
compared between the ovary and the testis, and during fetal development. No signal was found for the negative control RNA (stomach and limb buds), demonstrating the specificity of the hybridization with the cDNA probes. The variations in the GnRH (filter 1) and GnRH-R (filter 2) mRNA levels during fetal life are presented in Fig. 5. After stripping and rehybridization of filters (filter 1 with GnRH-R and filter 2 with GnRH cDNA probes), similar results were obtained (data not shown).

Some discrepancies appeared between the intensity of PCR signals and the dot blot results; however these do not reflect the true situation. Indeed, the RT-PCR protocol was not quantitative and the amplification may differ among the different samples, a difference that also appears on gel after amplification and on autoradiography after hybridization (Fig. 3). In contrast, the dot blot analysis is quantitative, and all values have been standardized to cyclophilin. In fetal testes, both GnRH and GnRH-R mRNA were detected as early as 14.5 dpc, consistent with our RT-PCR data. During early development until 19.5 dpc, testicular GnRH mRNA levels fluctuated around 0.3 arbitrary units, then increased markedly. GnRH-R mRNA levels followed a similar pattern with slight fluctuations until 19.5 dpc. During the last two days of gestation, both mRNAs increased dramatically.

In fetal ovaries, in contrast to our results with RT-PCR, no GnRH mRNA was detected by dot blot hybridization until 18.5 dpc. GnRH mRNA levels increased markedly between 17.5 and 18.5 dpc, reaching a maximum of about 1.25 arbitrary units at 19.5 dpc then maintaining a plateau until term. As in the testes, low levels of GnRH-R mRNA were detectable at 15.5 dpc and then progressively increased until 19.5 dpc, after which they paralleled GnRH mRNA levels until birth.

![Figure 3](image-url)  
**Figure 3** Agarose (1-2%) gel electrophoresis and Southern analysis of products obtained from RT-PCR of RNA extracted from fetal rat testes (A) and ovaries (B), using GnRH-R primers. I, gel stained with ethidium bromide; II, Southern blot hybridization using 32P-labeled oligonucleotide J. The expected size is indicated between A and B. M, DNA marker (HaeIII-digested PhiX-174); 14.5 to 21.5, fetal stages in days post-coitum; RT, control in which RNA was replaced by water during RT; PCR, control in which cDNA was replaced by water during PCR.

![Figure 4](image-url)  
**Figure 4** Cyclophilin mRNA levels in rat fetal testes (A) and ovaries (B). Total RNA from fetal gonads was assessed for its cyclophilin mRNA content by dot blot hybridization using a 32P-labeled cyclophilin cDNA probe. Autoradiograms were analyzed by densitometry. Data were standardized against the amount of total RNA loaded. Densitometric values expressed in arbitrary units (a.u.) are the results of two determinations of three diluted aliquots. Data were fitted to a linear regression with a slope and a correlation coefficient which were not statistically different from zero (P>0.05).
When gonadal GnRH and GnRH-R mRNA levels were compared between fetal testis and ovary, we noticed that GnRH and, to a lesser extent, GnRH-R mRNA levels appeared to increase earlier in the ovary than in the testis (18.5 vs 19.5 dpc). Furthermore, at 21.5 dpc the densitometric values for GnRH mRNA were equivalent in both sexes. By contrast, they were about 2.4 times higher in the testis than in the ovary after hybridization with the GnRH-R probe.

Discussion

The presence of GnRH receptors in Leydig cells (Clayton et al. 1980, Sharpe & Fraser 1980a) and in granulosa and luteal cells (Clayton et al. 1979, Harwood et al. 1980a,b, Reeves et al. 1980) has been supported by the identification of a mRNA with a sequence identical to that of the pituitary GnRH-R (Moumni et al. 1994). However, the exact nature of the endogenous GnRH-like material found in Sertoli cells (Sharpe & Fraser 1980b, Paull et al. 1981, Bhasin et al. 1983) and in granulosa cells (Aten et al. 1986) of adult rats remains uncertain.

In the present study using RT-PCR combined with Southern blotting, we have demonstrated the presence of a GnRH mRNA in the testis as well as in the ovary at all stages of development. After sequencing, we observed that adult rat testis transcript has a sequence identical to that of the hypothalamic GnRH-R (Moumni et al. 1994). However, the exact nature of the endogenous GnRH-like material found in Sertoli cells (Sharpe & Fraser 1980b, Paull et al. 1981, Bhasin et al. 1983) and in granulosa cells (Aten et al. 1986) of adult rats remains uncertain.

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(Goubau et al. 1992) because these transcripts could not be amplified using our set of primers. Taken together, these results indicate the presence of at least two populations of GnRH transcripts in the ovary.

In previous binding studies, the presence of GnRH-R was detected in the testis beginning one day after birth (Huhtaniemi et al. 1985). However, Habert et al. (1991) have shown that a GnRH agonist can exert a negative action on testicular steroidogenesis as early as 17·5–18·5 dpc. The present RT-PCR study detected a GnRH-R amplified fragment with a size identical to that of the adult form (Moumni et al. 1994) and recognized by a pituitary GnRH-R cDNA probe. As suggested for the adult rat, these data strengthen the idea that locally produced GnRH could operate as a paracrine or autocrine factor in the regulation of fetal gonadal functions through a specific receptor.

A prerequisite for evaluating a role for gonadal GnRH during fetal life is to study the ontogeny of expression of the GnRH and GnRH-R genes, with regard to age-dependent changes in cellular contents and in steroidogenesis. To this purpose, we investigated the presence of GnRH and GnRH-R mRNA in rat testis and ovary from 14·5 and 15·5 dpc respectively, when the morphological determination of the gonad is just completed, and in the testis, particularly when steroidogenic fetal Leydig cells would be differentiated (Byskov & Hoyer 1994, Ojeda & Urbanski 1994). We quantified GnRH and GnRH-R mRNA levels by dot blot hybridization of total RNA because this technique offers the advantages of comparing mRNA concentrations among different samples within one experiment, eliminating the variations in RNA loading by standardization to the same internal control. Densitometric values obtained with a labeled cyclophilin cDNA probe and standardized to total RNA, exhibited only slight fluctuations in the testis as well as in the ovary during fetal development, as attested by the regression analysis. This is consistent with the lack of regulation of cyclophilin expression in adult tissues (Danielson et al. 1988) and in the hypothalamus during postnatal development (Jakubowski et al. 1991). Thus, we decided that cyclophilin mRNA was an appropriate internal standard that could also be useful for future studies in fetal gonads.

Using RT-PCR and dot blot hybridization, we clearly demonstrated the presence of GnRH-R mRNA at 14·5 dpc in the testis and at 15·5 dpc in the ovary. By contrast, GnRH mRNA levels were quantified by dot blot from 18·5 dpc in the ovary, four days later than in the testis. However, using RT-PCR, GnRH mRNA was detected at 15·5 dpc. Such discrepancy between the dot blot and RT-PCR results may be explained by the high sensitivity of RT-PCR which allows the detection of mRNA in quantities under the limits of detection by dot blots, although the RT-PCR protocol we used in this study was not quantitative. Our present study revealed other differences between the ovary and the testis. In late development, the GnRH-R mRNA concentration was about 2·4 times higher in the testis than in the ovary. In contrast, in the adult, the GnRH-R mRNA content was lower in the testis than in the ovary (respectively 5% and 40% compared with the pituitary) (Kakar et al. 1994). This suggests a postnatal switch in the level of expression of the GnRH-R gene between the sexes. In addition, we found that while GnRH and GnRH-R mRNA concentrations follow a concomitant increase in the gonads, this increase appears to occur earlier in the ovary than in the testis (18·5 dpc vs 19·5 dpc). Taken together, these results demonstrate that in rat gonads, the ontogeny of expression of the GnRH and GnRH-R genes is coordinately regulated but in a sex-dependent manner.

When compared with the developmental onset of gonadotropin receptor (LH-R and FSH-R) gene expression (Sokka et al. 1992, Zhang et al. 1994, Rannikki et al. 1995) LH-R transcripts appeared one day after GnRH-R mRNA in Leydig cells (15·5 and 14·5 dpc respectively), whereas FSH-R transcripts were detected in Sertoli cells two days later than GnRH mRNA (16·5 and 14·5 dpc respectively). In the ovary, gonadotropin receptor genes were expressed post-partum in granulosa cells. Thus, the onset of GnRH and GnRH-R gene expression in fetal gonads may be independent of LH-R and FSH-R gene expression in the corresponding cells, and in all cases, gonadal expression of the GnRH and GnRH-R genes appeared to proceed, and thus also to be independent of, the initiation of LH and FSH secretion from the pituitary (Huhtaniemi 1995).

Treatment with GnRH agonists in vivo and in vitro have demonstrated an inhibitory effect of GnRH on Leydig cell steroidogenesis in immature and adult hypophysectomized rats (Bambino et al. 1980), and in decapitated rat fetuses (Habert 1992). In organotypic cultures of fetal testis, Habert et al. (1991) have also shown that a GnRH agonist inhibited basal testosterone production between 17·5–18·5 dpc and 20·5 dpc, and LH-stimulated testosterone production thereafter. These results suggest that GnRH-R mRNA is translated into a functional receptor protein very soon after its formation, but this cannot be verified by immunodetection, as antibodies against GnRH-R are not available at the present time. It is well documented that, in vivo, after reaching its maximum around 18·5 dpc, testosterone production by the testis remains unchanged (Warren et al. 1984), or decreases when it is expressed per cell (Picon 1976, Tapanainen et al. 1984, Saez 1994). During the same period, the density of cells producing testosterone per testis has been reported to be constant (Tapanainen et al. 1984, Kerr & Knell 1988, Saez 1994). Our finding of a marked increase in GnRH-R mRNA concentration suggests a stimulation of GnRH-R gene expression or an increase in the stability of GnRH-R mRNA. The decreasing steroidogenic activity of the Leydig cells is puzzling because of the concomitant rise in both the plasma concentration of LH (Huhtaniemi
et al. (1995) and the LH-R content (Warren et al. 1984). One possible explanation for this paradox is that some inhibitory paracrine/autocrine action may be established during late fetal life. The relationship between the marked increase in GnRH, as well as in GnRH-R, mRNA concentrations from 19.5 dpc to birth, and the decrease in Leydig cell activity, would be consistent with a potential implication of locally synthesized GnRH in such an inhibitory action. In addition, in cultured interstitial cells from the testes of hypophysectomized adult rats, GnRH agonists decrease testosterone production by an inhibitory effect on 17α-hydroxylase and 17,20-desmolase activities (Hsueh et al. 1983). In the fetal testis, the onset of the main steroidogenic enzyme genes expression, and particularly of 17α-hydroxylase, occurs around 15.5 dpc (Majdic et al. 1996), and thus early steroidogenesis, as GnRH and GnRH-R genes expression, is independent of gonadotropins (El-Gehani et al. 1998). We also point out that in the mouse, a species that lacks the gonadal GnRH-R (Wang et al. 1983), there is no decline in testosterone production in late fetal life (O’Shaughnessy et al. 1998).

In the adult rat ovary, in vivo and in vitro studies have demonstrated that, as in the testis, GnRH or GnRH agonists can affect gonadal functions. Indeed, GnRH has been shown to inhibit the expression of genes involved in steroidogenesis (Lynch et al. 1993, Richards 1994), and to modulate follicular response to gonadotropins, depending on the functional state of differentiation of follicular cells (Richards et al. 1987, Richards 1994, Srivastava et al. 1995). The autocrine actions of locally produced GnRH on steroidogenesis and/or folliculogenesis are difficult to assess in the fetal ovary, because these functions start a few days post-partum (Ojeda & Urbanski 1994, Huhtaniemi 1995). Moreover, there are no data reporting GnRH binding activity in the fetal ovary. In fact, granulosa cells are not yet organized in follicles (Byskov & Hoyer 1994, Ojeda & Urbanski 1994). However, as early as 13.5 dpc, the apparent precursors of theca and/or granulosa cells are able constitutively to express truncated LH-R mRNA (Sokka et al. 1996). Whether GnRH and GnRH-R mRNA are colocalized with these transcripts awaits further studies.

In conclusion, our study has demonstrated that the GnRH and GnRH-R genes are expressed in fetal gonads. The ontogeny of this expression underlined several similarities and differences between the sexes that encourage further investigation of the factors regulating GnRH and GnRH-R gene expression in gonadal tissues. Furthermore, our findings strengthen the previous hypothesis that gonadal GnRH could be involved in the age-related decrease of fetal Leydig cell activity and, post-partum, in the control of follicle differentiation in the ovary. Complementary studies, especially the ontogeny of expression of the GnRH and GnRH-R genes in postnatal gonads, will help us to evaluate further the role of GnRH as an intragonadal regulator.

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