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Differential regulation of leucine-rich primary response gene 1 (*LRPR1*) mRNA expression in rat testis and ovary

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In immature rat Sertoli cells, leucine-rich primary response gene 1 (*LRPR1*) represents a follicle stimulating hormone (FSH)-responsive gene; the function of the encoded protein is not yet known. *LRPR1* mRNA expression is up-regulated very rapidly and specifically by FSH, both in cultured Sertoli cells and *in vivo* in testicular tissue. In view of these properties of *LRPR1*, we have investigated *LRPR1* mRNA expression and its regulation in more detail, in testis and ovary of fetal, immature, and adult rats. In addition, we have studied the expression of FSH receptor (*FSHR*) mRNA in relation to *LRPR1* mRNA expression. In rat testis, *LRPR1* mRNA and *FSHR* mRNA followed a similar expression pattern, during postnatal development and also at different stages of the spermatogenic cycle in the adult rat. Furthermore, after short-term challenge of the FSH signal transduction pathway in intact immature rats by injection with a relatively high dose of FSH, an inverse relationship between *LRPR1* mRNA (up-regulation) and *FSHR* mRNA expression (down-regulation) was observed. Similar studies in the ovary provided completely different results. *LRPR1* mRNA in the postnatal ovary is present well before expression of *FSHR* mRNA can be first detected. In addition, incubation of ovaries of immature rats with FSH or dibutyryl cyclic AMP (dbcAMP) did not result in up-regulation of *LRPR1* mRNA expression. During fetal development, the *LRPR1* mRNA expression pattern involved many more tissues, in contrast to the relatively tissue-specific expression of *LRPR1* mRNA in gonads of 21 day old and adult rats. Moreover, *LRPR1* mRNA expression could be detected as early as 12.5 days post-coitum, whereas *FSHR* mRNA is absent at this stage of fetal development. We concluded that the pronounced regulation of *LRPR1* by FSH observed in the immature rat testis does not occur in the ovary. Furthermore, in the ovary *LRPR1* mRNA expression does not appear to be dependent on FSH action. Finally, the *LRPR1* gene product may play a general role during fetal development.

Key words: FSH receptor/ovary/reproduction/spermatogenesis/testis

Introduction

Follicle stimulating hormone (FSH), a glycoprotein hormone that is produced in the pituitary gland, plays a major role in gonadal development and function. In the ovary, FSH is involved in control of proliferation of granulosa cells and selection of dominant follicles (Chappel and Howles, 1991; Wilson and Foster, 1992a; Richards, 1994), and in the testis FSH controls proliferation, differentiation, and maturation of Sertoli cells. It is generally thought that FSH and testosterone are the main hormonal regulators of spermatogenesis (Means *et al.*, 1976; Dym *et al.*, 1979; Russell *et al.*, 1987; Wilson and Foster, 1992b). The recent development of an FSHβ knockout mouse has improved our understanding of the relative importance of FSH. In the male knockout mice, spermatogenesis can proceed completely, but sperm count and testis size were decreased considerably (Kumar *et al.*, 1997). Female FSHβ knockout mice were completely infertile, and follicle development did not proceed beyond the pre-antral stage in these animals, indicating the absolute dependence of the later stages of follicle development on FSH during the ovarian cycle (Kumar *et al.*, 1997).

Upon binding of FSH to the FSH receptor (FSHR), which is only expressed in testicular Sertoli cells and in the granulosa cells of ovarian follicles, the GTP-binding protein G_s is activated, eventually resulting in activation of adenylyl cyclase and production of cyclic AMP (cAMP). The second messenger, cyclic AMP (cAMP), then activates cAMP-dependent protein kinase A (Casey and Gilman, 1988; Reichert and Dattatreyamurty, 1989). FSH regulates transcription of many genes and synthesis of many proteins through the cAMP pathway. Well-known examples are α-inhibin (Toebosch *et al.*, 1988; Klaij *et al.*, 1990), androgen binding protein (Reventos *et al.*, 1988; Hall *et al.*, 1990), c-*fos* (Hall *et al.*, 1988), and aromatase (Fitzpatrick and Richards, 1991). Recently, we cloned a new FSH-responsive gene from cultured immature rat Sertoli cells, and named this gene leucine-rich primary response gene 1 (*LRPR1*) (Slegtenhorst-Eegdeman *et al.*, 1995). *LRPR1* mRNA expression in the immature rat testis is very strongly and rapidly up-regulated by FSH, both *in vitro*

(in cultured Sertoli cells) and *in vivo* (after injection of exogenous FSH in immature intact rats) (Slegtenhorst-Eegdeman *et al.*, 1995).

Expression of several genes occurs not only in response to FSH stimulation, but also to other hormones. For example, c-*fos* mRNA expression in cultured Sertoli cells is also regulated by fibroblast growth factor (FGF) or the testicular paracrine factor, peritubular modulatory substance (PModS) (Smith *et al.*, 1989; Norton and Skinner, 1992) and α-inhibin mRNA expression is up-regulated when Sertoli cells are cultured in germ cell-conditioned medium in the absence of FSH (Pineau *et al.*, 1990). However, until now, no other such regulators of *LRPR1* mRNA expression have been identified. Since *LRPR1* mRNA expression is not only very rapidly but also specifically regulated by FSH in Sertoli cells, expression of *LRPR1* mRNA seemed to be a useful parameter for evaluation of testicular FSH action, both *in vivo* and *in vitro*.

In the adult rat, expression of *LRPR1* mRNA is found not only in the testis but also at a lower level, in ovary, spleen, brain, and lung. Regulation of *LRPR1* mRNA expression by FSH in immature rat Sertoli cells was found to be independent of protein synthesis, but appeared to be absent in the presence of a transcription inhibitor, and we concluded that *LRPR1* is a primary response gene to FSH (Slegtenhorst-Eegdeman *et al.*, 1995).

Following the cloning of rat *LRPR1* (Slegtenhorst-Eegdeman *et al.*, 1995), the isolation of a human homologue of this gene has been reported. The human *LRPR1* gene is mapped to the X chromosome at Xq22, and the encoded protein shows 72% homology at the amino acid level with the rat protein (Roberts *et al.*, 1996). Interestingly, the yeast gene *mis6*$^{+}$ and its protein product Mis6, show a weak but significant similarity to the rat *LRPR1* cDNA and amino acid sequences. Mis6 appears to be involved in equal segregation of sister chromosomes during mitosis (Saitoh *et al.*, 1997).

In the present paper, we describe experiments on the developmental regulation of *LRPR1* mRNA expression in ovary, testis and other tissues in the context of FSH regulation of gonadal activity. *FSHR* mRNA expression and the short-term down-regulation of receptor mRNA by FSH (Themmen *et al.*, 1991) were used as parameters to determine organ-sensitivity to FSH. The expression of *LRPR1* mRNA in gonadal and non-gonadal tissues and the gonadal regulation by FSH, were determined during postnatal gonadal development and in the adult rat.

Materials and methods

Animals and treatments

Wistar rats were maintained under standard animal house conditions. Testicular *FSHR* mRNA and *LRPR1* mRNA expression was determined at different ages and also after i.p. injection with 0.15 IU/g bodyweight human FSH (Metrodin; Serono, Geneva, Switzerland). Seminiferous tubule segments at defined stages of the spermatogenic cycle were collected according to Parvinen *et al.* (1982). For in-vitro treatment of rat ovaries, ovaries of 30 day old rats were collected, bisected and incubated in 1 ml of M199 medium with Earle's salts and L-glutamine (Gibco BRL, Gaithersburg, MD, USA) supplemented with 2.2 mg/ml $NaHCO_3$ and 5.95 mg/ml HEPES (pH 7.2). Incubation took place for 4 or 8 h at 37°C under 5% CO_2/95% O_2, in the presence or absence of 0.5 mM dibutyryl-cyclic AMP (dbcAMP; Boehringer Mannheim, Mannheim, Germany) or 1000 mIU/ml recombinant human FSH (rhFSH; Organon NV, Oss, The Netherlands). Each incubation was performed in duplicate with two ovaries per incubation. After the incubation, the medium was used to measure progesterone production and the ovaries were used to determine *LRPR1* mRNA and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) mRNA expression.

RNA isolation and RNase protection assay

Total gonadal and fetal RNA was isolated using the LiCl/Urea method (Auffray and Rougeon, 1980). Rat *FSHR* anti-sense cRNA probes were generated from a 364 bp *Eco*RV/*Nco*I fragment corresponding to bp 775–1139 of the rat *FSHR* gene, subcloned in pBluescript KS (Stratagene; Westburg, Leusden, The Netherlands) using T7 RNA polymerase (Stratagene) and α-[^{32}P]-UTP. *LRPR1* anti-sense cRNA probes were generated from a 322 bp *Bam*HI/*Bgl*II fragment corresponding to bp 1769–2091 of the rat *LRPR1* gene, subcloned in pBluescript KS using T7 RNA polymerase and α-[^{32}P]-UTP. A 113 bp rat *GAPDH* probe corresponding to bp 197–310 of the rat *GAPDH* gene (Fort *et al.*, 1985) was used to determine the relative amount of RNA loaded on the gel. For the RNase protection assay depicted in Figure 3, a larger *GAPDH* probe (bp 1–310) was used. Depending on the *GAPDH* probe used, double or multiple bands were obtained in the RNase protection assay. These are the results of internal cleavage of the RNA duplexes or of secondary structures formed in the probe. Total gonadal RNA (5 or 10 μg) was analysed by RNase protection assay according to Sambrook *et al.* (1989). For the experiments on fetal *LRPR1* mRNA expression, 1.5% of the total amount of the RNA isolated per fetus was used, supplemented with tRNA (Boehringer Mannheim) to a total of 50 μg. This was done in order to load proportional fractions of gonadal RNA. We postulated that *LRPR1* is only present in the gonads of the rat fetus. This type of presentation would give us insight in the ontogeny of *LRPR1* mRNA expression in the rat gonad. For days 12.5 and 13.5 post-coitum, bands were only visible on an overexposed X-ray or by using a phosphor screen (Molecular Dynamics; B&L Systems, Zoetermeer, The Netherlands). Therefore, a quantitative analysis of the *LRPR1* mRNA level (ratio *LRPR1*/*GAPDH*) was also given (Figure 5).

The relative amount of protected mRNA fragments was quantified through exposure of the gels to a phosphor screen (Molecular Dynamics), followed by calculation of the relative density of the obtained bands using a phospho-imager and ImageQuant analysis software (Molecular Dynamics). For the *GAPDH* mRNA patterns, all bands were included. The ratios between the arbitrary units obtained for the *LRPR1*, *FSHR* and *GAPDH* mRNAs were determined.

All RNAse protection assays were performed two or three times. In the figures, one representative RNAse protection assay and the graphs belonging to that assay are shown.

Measurement of progesterone

The concentration of progesterone in the medium in which ovaries had been incubated, was measured by radioimmunoassay. The values were corrected for procedural losses (recovery 70–80%). The progesterone antibody was raised against 11α-OH-progesterone–hemi-succinate–bovine serum albumin (BSA) complex, and data on the specificity of the antibody have been described earlier (de Jong *et al.*, 1974). The intra- and interassay coefficients of variation of the progesterone assay were 10 and 5% respectively.

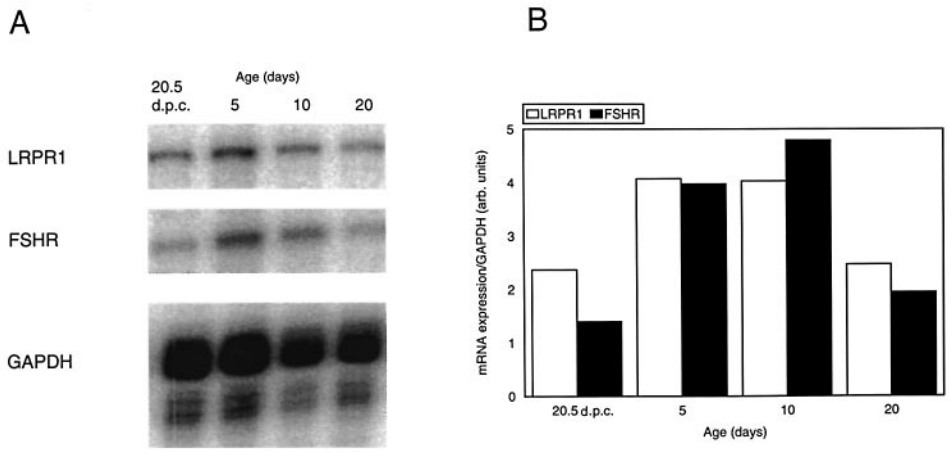

Figure 1. Leucine-rich primary response gene 1 (*LRPR1*) mRNA and follicle stimulating hormone receptor (*FSHR*) mRNA expression in immature rat testis. Total RNA was isolated from testes of rats of different ages, and subjected to RNase protection assay with *FSHR*, *LRPR1*, and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) cRNA probes. Subsequently, the *FSHR*/*GAPDH* mRNA and the *LRPR1*/*GAPDH* mRNA ratios were determined as described in Materials and methods. (**A**) Results of the RNase protection assay. LRPR1, FSHR and GAPDH indicate the positions of the respective protected fragments. d.p.c. = days post-coitum. (**B**) Quantitative analysis of the *LRPR1*/*GAPDH* (open bars) and the *FSHR*/*GAPDH* (black bars) mRNA ratios. p.c. = post-coitum.

Results

LRPR1 and FSHR mRNA expression in the testis

Expression of *LRPR1* and *FSHR* mRNAs was determined using RNase protection assay on total rat testis RNA at 20.5 days post-coitum (1 day before birth) and at days 5, 10 and 20 after birth (Figure 1). The expression of both *LRPR1* mRNA and *FSHR* mRNA was highest at days 5 and 10 of postnatal life. These results indicate that, in the developing testis, *LRPR1* mRNA is correlated with *FSHR* mRNA expression. A decrease in the relative size of the somatic cell compartment of the testis during initiation of spermatogenesis contributes to the lower level of *LRPR1* and *FSHR* mRNA expression at day 20.

The influence of interaction with germ cells at different stages of the spermatogenic cycle on Sertoli cell *LRPR1* mRNA expression was studied in adult rat testis. Using the transillumination-assisted microdissection technique (Parvinen and Ruokonen, 1982), segments of seminiferous tubules at specific stages of the spermatogenic cycle were isolated, pooled, and subjected to RNase protection analysis of *LRPR1* mRNA and *FSHR* mRNA expression (Figure 2). It was observed that both mRNAs show a similar expression pattern (levels are lowest at stages VI to VIIab of the cycle and increase to a maximum at stages XIII to I), although the changes in *LRPR1* mRNA expression are not as pronounced as those observed for *FSHR* mRNA. Similar to the results found in the developing testis, the expression patterns of *LRPR1* mRNA and *FSHR* mRNA are related during the spermatogenic cycle.

Taken together, the results indicate a causal relationship between FSH activity and *LRPR1* mRNA expression. Therefore we tested the response of *LRPR1* mRNA expression to a short-term stimulation with FSH. Rats of different ages (10, 15 and 20 days old and adult) received i.p. injections of human FSH. We have previously shown that incubation of Sertoli cells with FSH results in a marked decrease in *FSHR* mRNA expression

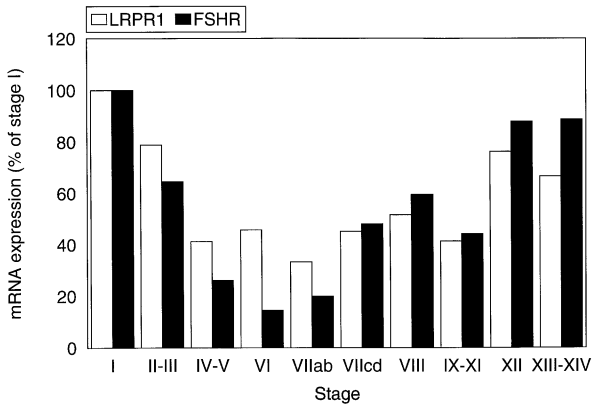

Figure 2. Leucine-rich primary response gene 1 (*LRPR1*) mRNA and follicle stimulating hormone receptor (*FSHR*) mRNA expression in isolated tubules at defined stages of the spermatogenic cycle. Total RNA was isolated from the tubule segments and subjected to RNase protection assay with *FSHR* and *LRPR1* cRNA probes. The level of mRNA expression was determined as described in Materials and methods and plotted as a percentage of the level of expression at stage I.

within 4 h as a result of FSH-induced destabilization of *FSHR* mRNA (Themmen *et al.*, 1991). In the present experiments, testicular *LRPR1* mRNA expression and *FSHR* mRNA expression were measured 4 h after FSH injection by RNase protection assay (Figure 3). In rats aged 15–20 days, and in adult rats, *LRPR1* mRNA expression responded well to FSH treatment, showing a 2–3-fold increase in expression level. Concomitantly, the level of *FSHR* mRNA expression was markedly decreased (Themmen *et al.*, 1991). The results in Figure 3 show that, in rat testis, *LRPR1* mRNA expression and FSH-sensitivity determined by *FSHR* mRNA expression are directly related, demonstrating an inverse relationship between *LRPR1* mRNA expression and *FSHR* mRNA expression. In contrast to the results obtained with 15 and 20 day old and adult rats, treatment of 10 day old rats did not yield a consistent response. In some animals both the *LRPR1* mRNA expression was increased and

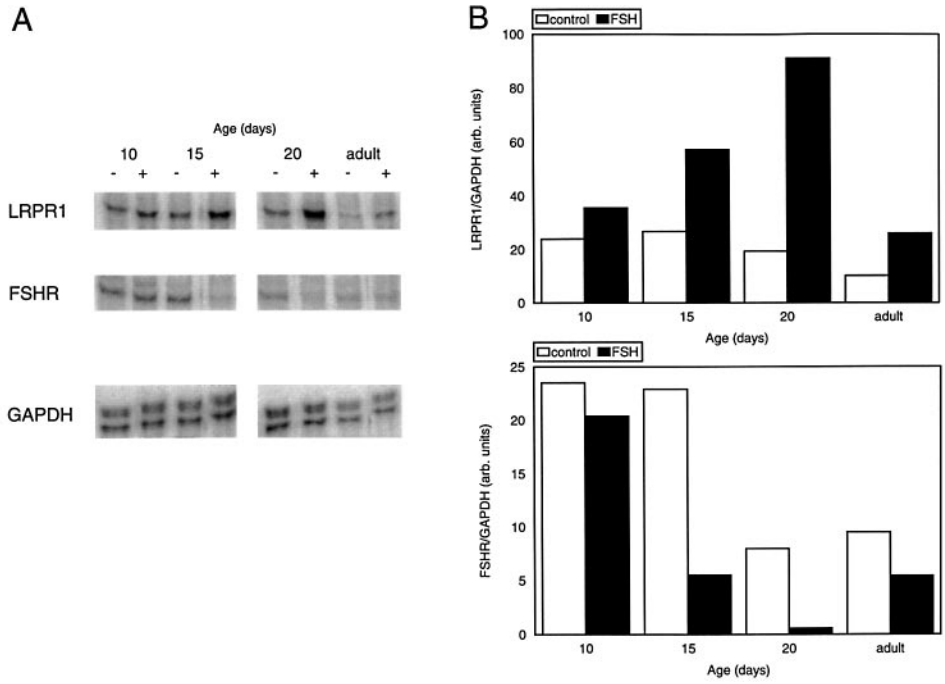

Figure 3. Leucine-rich primary response gene 1 (*LRPR1*) mRNA and follicle stimulating hormone receptor (*FSHR*) mRNA expression in response to treatment with exogenous FSH during testis development. Male rats of 10, 15 and 20 days old and adult, received i.p. injections of either saline (– or open bars) or 0.15 IU/g bodyweight of human urinary FSH (+ or black bars), and the testes were collected after 4 h. Total testicular RNA was isolated and subjected to RNase protection assay with *LRPR1*, *FSHR* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) cRNA probes. Subsequently, the *LRPR1*/*GAPDH* mRNA ratio (upper graph) and the *FSHR*/*GAPDH* ratio (lower graph) were determined as described in Materials and methods. (**A**) Results of the RNase protection assay. LRPR1, FSHR and GAPDH indicate the positions of the respective protected fragments. (**B**) Quantitative analysis of the *LRPR1*/*GAPDH* mRNA and the *FSHR*/*GAPDH* mRNA ratios.

FSHR mRNA was decreased in response to FSH, whereas in other animals no change was observed. Small differences in the developmental stage of these young animals may have caused this variation and we did not investigate this further.

LRPR1 and FSHR mRNA expression in the ovary

Similar experiments as described for the testis were performed using female rats to investigate the regulation of ovarian *LRPR1* mRNA expression in relation to *FSHR* mRNA expression and FSH sensitivity. Determination of *LRPR1* and *FSHR* mRNA expression in developing ovaries at 20.5 days post-coitum, and in 1, 3, 21, 25, and 30 day old rats (Figure 4), revealed a completely different pattern to that observed in the testis. Ovarian *FSHR* mRNA expression was very low immediately before and after birth, but increased significantly at day 21. However, no considerable change in the level of *LRPR1* mRNA expression was observed. Moreover, *LRPR1* mRNA was already present at an early stage of ovarian development, when the ovaries did not (or virtually not), express *FSHR* mRNA. These results indicate that *LRPR1* mRNA expression in the ovary is, at least partially, independent of FSH.

The results described above point to a mechanism of ovarian regulation of *LRPR1* mRNA expression that is independent of FSH and the cAMP pathway. FSH- and cAMP-sensitivity of ovarian *LRPR1* mRNA expression were tested by incubating isolated bisected ovaries of 30 day old rats in the absence or presence of dbcAMP or recombinant human FSH (rhFSH). After 4 or 8 h of incubation, ovarian RNA was isolated and *LRPR1* mRNA was determined. Progesterone determination in the medium served as a control for dbcAMP or FSH action (Table I). Although incubation with FSH did result in an increased level of progesterone production, *LRPR1* mRNA expression was not affected. Progesterone production was increased to a relatively high level in ovaries incubated with dbcAMP, which may largely reflect a direct effect of dbcAMP on the theca cells. Also dbcAMP treatment did not result in an effect on *LRPR1* mRNA expression. These results indicate that in the ovary, there is no short-term up-regulation of *LRPR1* mRNA expression by dbcAMP or FSH.

Fetal LRPR1 mRNA expression

To determine the ontogeny of fetal *LRPR1* mRNA expression in relation to *FSHR* mRNA expression, total RNA was isolated from fetuses at different stages of development. Subsequently, proportional fractions of each of the RNA isolates were subjected to RNase protection assay (Figure 5). Since *FSHR* mRNA was first detected at day 16.5 post-coitum in male rats and at postnatal day 1 in females (Rannikko *et al.*, 1995), we expected *LRPR1* mRNA expression to become detectable around the same time in development. Surprisingly, with an overexposure of the radiogram, *LRPR1* mRNA was detected as early as day 12.5 post-coitum (not shown). *LRPR1* expression per μg of total RNA (ratio *LRPR1*/*GAPDH*) was highest at early fetal development (Figure 5). From day 14.5 post-coitum onwards, the level of *LRPR1* mRNA expression was relatively stable and no difference in the level of *LRPR1*

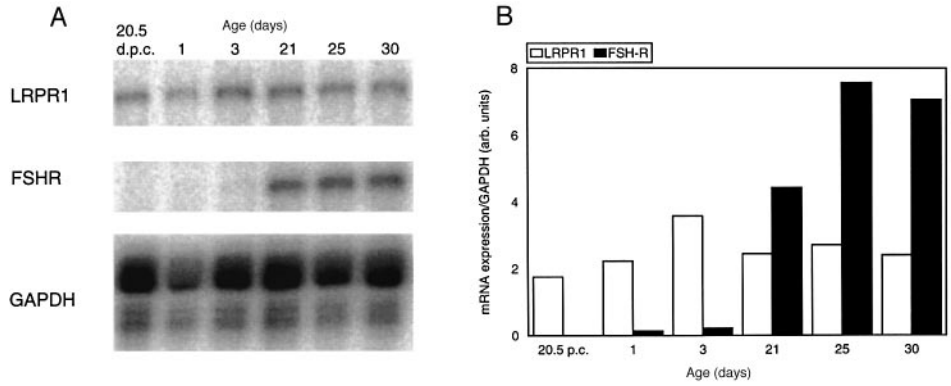

Figure 4. Leucine-rich primary response gene 1 (*LRPR1*) mRNA and follicle stimulating hormone receptor (*FSHR*) mRNA expression in the immature ovary. Total RNA was isolated from ovaries of rats of different ages and subjected to RNase protection assay with *LRPR1*, *FSHR*, and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) cRNA probes. Subsequently, the *LRPR1*/*GAPDH* mRNA and the *FSHR*/*GAPDH* mRNA ratios were determined. (**A**) Results of the RNase protection assay. LRPR1, FSH-R and GAPDH indicate the positions of the respective protected fragments. d.p.c. = days post-coitum. (**B**) Quantitative analysis of the *LRPR1*/*GAPDH* (open bars) and the *FSHR*/*GAPDH* (black bars) mRNA ratios.

Table I. Effect of dibutyryl cyclic AMP (dbcAMP) and follicle stimulating hormone (FSH) on leucine-rich primary response gene 1 (*LRPR1*) mRNA expression in rat ovary during in-vitro incubation. The results of two individual samples are given

Treatment		*LRPR1* (%)[a]	Progesterone (pg/ml)
Control	4 h	100	136/152
dbcAMP		104/106	1134/1190
FSH		84/122	199/245
Control	8 h	100	139/203
dbcAMP		86/130	1297/1443
FSH		99/ND	380/752

[a]*LRPR1*/glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) mRNA ratio; control levels were set at 100%. Ovaries were incubated for the indicated time periods in the absence or presence of 0.5 mM dbcAMP or 1000 mIU/ml human recombinant FSH.
ND = no data available.

mRNA expression between the sexes was observed (not shown). Surprisingly, the levels at days 12.5 and 13.5 post-coitum are similar to those found in testis from FSH-treated 21 day old rats. These results point to extra-gonadal expression of *LRPR1* mRNA in the developing fetus. Indeed, considerable expression of *LRPR1* mRNA was found in RNA isolated from fetal brain, kidney, and liver at day 19.5 post-coitum (data not shown). Thus, in contrast to the relatively gonad-specific expression found in the adult rat, with a very low level of *LRPR1* mRNA expression in adult brain, spleen, and lung (Slegtenhorst-Eegdeman *et al.*, 1995), fetal expression appears to be less organ-specific. *FSHR* mRNA expression was undetectable at all fetal ages, when whole fetus RNA was analysed, despite the very sensitive RNase protection assay (data not shown). The small percentage of testicular RNA present in the samples may be the cause of this lack of detection, and a polymerase chain reaction (PCR)-based method might provide semi-quantitative data to support this.

Discussion

In the present study, *FSHR* mRNA expression and FSH-induced down-regulation of *FSHR* mRNA were determined, in relation to *LRPR1* mRNA expression. In the male, *LRPR1* mRNA expression appears to be correlated with both *FSHR* mRNA expression and FSH sensitivity of the testis. In contrast, the present data show that ovarian cells express *LRPR1* mRNA before they can respond to FSH, at day 20.5 post-coitum and at later ages, and no relationship between FSH response and *LRPR1* mRNA expression was observed. Although it was initially thought that *LRPR1* mRNA expression was largely gonad-specific, initial investigation of fetal tissues revealed expression of this mRNA in many other tissues.

During postnatal development of the testis, Sertoli cells differentiate and the *FSHR* mRNA level is increased (Figure 1) (Rannikko *et al.*, 1995), paralleled by an increase in *LRPR1* mRNA. During testis development, the number of Sertoli cells increases under the influence of FSH as a mitogenic agent (Griswold *et al.*, 1977; Ultee-van Gessel *et al.*, 1988; Arslan *et al.*, 1993; Meachem *et al.*, 1996). Between days 15 and 20, the Sertoli cells cease to divide (Steinberger and Steinberger, 1971; Orth, 1982; Van Haaster *et al.*, 1992) and the number of germ cells increases rapidly as spermatogenesis proceeds. The growing number of germ cells, in particular in the period 2–4 weeks after birth, markedly contributes to a decrease in the relative level of *FSHR* mRNA and *LRPR1* mRNA expression, since both genes are only expressed in Sertoli cells.

The present results indicate that in immature rats responsiveness of Sertoli cells to FSH increases with age. However, in terms of both *FSHR* mRNA down-regulation and *LRPR1* mRNA up-regulation, the response to FSH in the adult testis was less pronounced compared with that in immature rats. Van Sickle *et al.* (1981) and Eskola *et al.* (1993) showed that FSH-stimulated cAMP production varied during testis maturation, being maximal around 10 days after birth and decreasing with age. However, for *LRPR1*, we observed maximal FSH responsiveness in 20 day old rats. Van Sickle *et al.* (1981) used Sertoli cell-enriched testes to determine FSH-responsiveness. Since germ cells influence Sertoli cells (Castellon *et al.*, 1989; Skinner, 1991), the discrepancy between our findings and those described by Van Sickle *et al.* (1981) could be caused by the presence of germ cells in the present

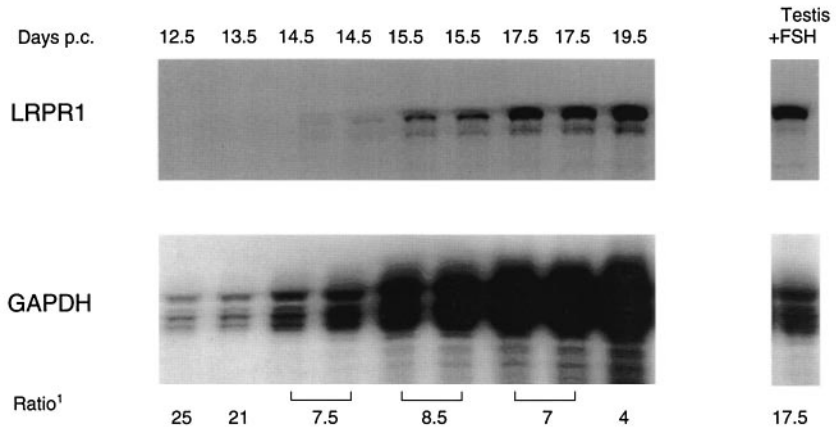

Figure 5. Ontogeny of leucine-rich primary response gene 1 (*LRPR1*) mRNA expression during fetal development. Total RNA was isolated from rat fetuses of different ages, and an equal percentage of total RNA per fetus was subjected to RNase protection assay using *LRPR1* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) cRNA probes. Subsequently, the relative amount of protected mRNA fragments were determined as described in Materials and methods. LRPR1 and GAPDH indicate the positions of the respective protected fragments. p.c. = days post-coitum. [1]Quantitative analysis of the *LRPR1* mRNA level, per μg of total RNA per fetus, given in arbitrary units per μg of RNA. For comparison, the level of *LRPR1* mRNA expression in total testis RNA isolated from 21 day old rats injected with human follicle stimulating hormone (FSH) was also included in this figure.

experiments. Eskola *et al.* (1993) used intact rats, but they did not investigate FSH-responsiveness between day 11 and day 30; FSH-induced cAMP production in 20 day old rats could still be higher than the level observed at day 11.

During the different stages of the spermatogenic cycle, *FSHR* mRNA expression is maximal at stages XII–I. These stages concern the meiotic divisions, very early spermiogenesis and spermatid nuclear condensation. Lowest *FSHR* mRNA expression was found at stages VI–VIIab, just prior to the release of the condensed spermatids into the lumen of the seminiferous tubules (Russell and Griswold, 1993). This pattern of *FSHR* mRNA expression is very similar to the pattern of FSH binding at different stages in the tubules (Kangasniemi *et al.*, 1990). *LRPR1* mRNA expression follows the expression level of *FSHR* mRNA, although the relative change of *LRPR1* mRNA expression is less pronounced than that of *FSHR* mRNA. In conclusion, it appears that, both in the immature and in the adult rat testis, *LRPR1* mRNA expression is related to *FSHR* mRNA expression.

Ovarian *LRPR1* mRNA expression does not appear to be directly related to FSH action. *FSHR* mRNA expression was first detected around day 3 after birth. This is consistent with an earlier report by Rannikko *et al.* (1995), who showed *FSHR* mRNA expression in rats from day 1 after birth, using a very sensitive reverse transcription (RT)–PCR-based method. However, *LRPR1* mRNA was already relatively highly expressed in the rat ovary at postnatal day 1. Furthermore, no increase of *LRPR1* mRNA was observed after postnatal day 3, when FSH binding to the FSHR can be detected first (Warren *et al.*, 1984). Moreover, the increase in the level of *FSHR* mRNA (present results), or the increased FSH binding to granulosa cells found from postnatal day 17 onwards (Uilenbroek and van der Linden, 1983), did not result in any change in *LRPR1* mRNA expression level. Therefore, it appears that ovarian *LRPR1* mRNA expression in immature female rats is independent of FSH activty.

Short-term regulation of *LRPR1* mRNA expression in granulosa cells was investigated *in vitro*. No change in *LRPR1* mRNA level could be discerned, although the incubated ovaries were perfectly capable of responding to dbcAMP or FSH with an increase in progesterone production. We also investigated *LRPR1* mRNA expression in ovaries of rats injected with pregnant mare serum gonadotropin (PMSG), but in that experiment, there was no significant difference in *LRPR1* mRNA expression (not shown).

After completion of the studies described in this paper, Saitoh *et al.* (1997) described the characterization of *mis6*$^+$, a gene isolated from the fission yeast *Schizosaccharomyces pombe*. The protein product Mis6 shows a relatively high homology with LRPR1 (27% homology in 361 amino acids overlap). During chromosome separation Mis6 protein acts at the end of the G_1 phase or at the onset of the S phase. The protein is located on the centromeres throughout the cell cycle, and appears to be required for maintenance of the structure of the inner centromere chromatin. Furthermore, Mis6 is involved in positioning of the centromeres and is required for establishment of a correct orientation of sister centromeres in metaphase cells. In the mis6-302 yeast strain, carrying a defective *mis6*$^+$ gene, positioning of the centromeres does not occur normally. As a consequence, unequal segregation of sister chromatids during mitosis was observed, resulting in large and small daughter nuclei. Furthermore, cell viability was decreased, compared to the wild type yeast strain (Saitoh *et al.*, 1997). The substituted amino acid residue in Mis6 which caused the mutation phenotype is also conserved in the rat and human LRPR1 proteins. In view of the homology of Mis6 and LRPR1, it is tempting to suggest that LRPR1 might also be involved in mitosis. However, this remains to be investigated, and the precise role of LRPR1 in mammalian development and gonadal function might be unrelated to control and progress of mitotic divisions. Since *LRPR1* mRNA expression in the rat testis is maximal around day 21 after birth, and Sertoli cells cease to

divide at ~day 15, it is unlikely that LRPR1 plays a role in mitotic proliferation in Sertoli cells, other than that it might be involved in mitotic silencing. During the spermatogenic cycle, highest levels of *FSHR* mRNA expression, FSH binding, and *LRPR1* mRNA expression are found at the stages that contain different types of undifferentiated type A spermatogonia undergoing mitotic proliferation. Furthermore, spermatocytes in different stages of the meiotic prophase and undergoing meiotic divisions are found. However, *LRPR1* could not be detected in spermatocytes (Slegtenhorst-Eegdeman *et al.*, 1995); the possible presence of *LRPR1* mRNA in spermatogonia has not yet been evaluated.

During fetal development, *LRPR1* mRNA expression is clearly not gonad-specific. The very high expression of *LRPR1* during fetal life may be related to a possible role of the protein in highly proliferating tissues, and this would be consistent with homology between *LRPR1* and the yeast *Mis6* gene product.

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