Pergamon

0960-0760(95)00049-6

J. Steroid Biochem. Molec. Biol. Vol. 53, No. 1–6, pp. 215–218, 1995

0960-0760/95 $9.50 + 0.00

The Molecular Biology of the FSH Receptor

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The actions of follicle stimulating hormone (FSH) mediated through its receptor are necessary for the proper functioning of mammalian gonads. The FSH receptor is localized on granulosa cells of the ovary and Sertoli cells of the testis. The expression of the FSH receptor (FSHR) in Sertoli cells varies *in vivo* as a function of the stage of the cycle of the seminiferous epithelium and in culture as a result of the addition of exogenous hormones. The gene for the FSH receptor is large and has been shown to be related in structure to the genes for luteinizing hormone (LH) receptor and thyroid stimulating hormone (TSH) receptor. The promoter region of the gene for FSHR does not contain a TATA box and has multiple transcriptional start sites. Less than 280 bp of the promoter are sufficient in transient transfection assays to direct expression of the chloramphenicol acetyl transferase gene (CAT) in a number of different cell types including non-gonadal cells. However, the promoter does direct the expression of a marker gene only into testis and ovary of transgenic mice.

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INTRODUCTION

Follicle stimulating hormone (follitropin; FSH) is a pituitary glycoprotein hormone that is necessary for normal reproduction in both male and female mammals. Sertoli cells and granulosa cells are the target cells for the action of FSH in the testes and ovaries, respectively. Sertoli cells provide physical and biochemical support for germ cell development [1, 2]. Granulosa cells in the ovary play a role in the overall support of the ovum and the development of the follicle [3]. According to this scenario FSH indirectly influences spermatogenesis and oogenesis by exerting influences on the corresponding somatic cells.

The granulosa cells of the ovary and the Sertoli cells of the testis share a number of similarities which have been noted previously [4]. Both cell types are necessary for the development and survival of the germ cells prior to ovulation or spermatogenesis. Immature and mature granulosa and Sertoli cells have some similar biochemical properties including the synthesis of some of the same proteins such as plasminogen activator and inhibin. Both granulosa cells and immature Sertoli cells are capable of synthesizing estrogens from an exogenous source of androgens [4].

Proceedings of the IX International Congress on Hormonal Steroids, Dallas, Texas, U.S.A., 24–29 September 1994.
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FSH RECEPTOR (FSHR)

Granulosa and Sertoli cells are target cells for the action of FSH and they are the only cell types which are thought to express the FSH receptor. The follicle stimulating hormone receptor (FSHR), like the receptors for luteinizing hormone (LH)/CG and thyroid stimulating hormone (TSH), is a member of a superfamily of receptors which act via interactions with G-proteins [5–8]. All of the receptors in this superfamily traverse the plasma membrane with seven highly conserved α-helices oriented with an extracellular amino terminus and an intracellular carboxy terminus [9, 10]. The glycoprotein hormone receptors, LH, FSH and TSH represent a small subclass of this superfamily that have the largest amino terminal domains. The cDNA for the FSH receptor was shown to encode a 675 amino acid, 75,000 Da protein with a 348 amino acid external domain [8]. The extracellular domains of the glycoprotein hormone subfamily share moderate sequence similarity that includes a repeated series of conserved 25 residue leucine-rich motifs, (for review see [11]).

FSHR GENE

We have characterized the FSHR gene and its promoter [12]. The FSHR gene is very large, encompassing at least 85 kb of DNA which is divided into 10 exons often separated by very large introns. The first 9 exons are relatively small and code for the amino

terminal extracellular domain which contains the leucine repeat regions. The region of the molecule which encodes the seven transmembrane spanning regions is contained within a single large exon (exon 10). The genes coding for FSHR, TSHR and LHR have a number of similarities: (1) they are all very large genes with 10 (TSHR, FSHR) or 11 (LH) exons; (2) the last exon in each gene codes for the entire membrane spanning region; and (3) exons 2–9 code for the amino terminal repeats. This structure suggests that these genes arose from a single gene in which an exon coding for the leucine repeats was duplicated several times and subsequently was combined with the conserved exon for the transmembrane region. It has been proposed that these receptors (FSHR, LHR and TSHR) constitute a closely related subfamily of the G-protein coupled receptors which have a relatively recent evolutionary origin [11].

EXPRESSION OF FSHR IN SERTOLI AND GRANULOSA CELLS

The cDNA probe for the FSH receptor has been used to localize the sites of synthesis and to examine the steady state levels of FSHR mRNA. Sertoli cells, ovary and testis were found to contain two major transcripts of 2.6 and 4.5 kb which hybridized to the FSH receptor DNA sequence [13, 14]. Using probes from the genomic sequence we were able to show that the 4.5 kb transcript has an extended 3′ terminus probably resulting from a read through of the first poly A signal sequence. Others have reported that ovarian mRNA analyzed on Northern blots had two major FSHR transcripts of 7.0 and 2.5 kb [15] or 2.6 and 5 kb [16]. *In situ* hybridization has been used to localize the FSH receptor mRNA exclusively in the granulosa cells in the ovary [16].

In the adult male rat, the expression of the FSHR undergoes a cyclic change in steady state levels [13]. Spermatogenesis in the adult rat is organized into a series of 14 stages which constitute the cycle of the seminiferous epithelium and are defined by the germinal cell composition of the tubules [17]. In the normal rat the 14 stages are present simultaneously at different regions along the length of the tubule and the release of spermatozoa (spermiation) which occurs at stage VIII is asynchronous. There is good evidence that the functions of the Sertoli cells vary with the different stages of the cycle. We have utilized a scheme of retinol deprivation and repletion which results in the synchronization of the testis to 3 or 4 related stages of the cycle [18, 19]. Spermatogenesis appears to proceed normally but since the entire testis is in roughly the same stages, spermiation occurs only every 12 to 13 days. We have utilized the retinol synchronization method to obtain synchronized testes which represent all parts of the cycle. This method allows the collection of sufficient amounts of stage synchronized testis to enable the analysis of low abundance cell products [20]. Utilizing the FSH receptor cDNA probe and mRNA isolated from synchronized testes we were able to measure the steady state levels of FSHR mRNA during the different stages of the cycle of the seminiferous epithelium [13]. We found that the relative levels of FSHR mRNA varied in a cyclic manner with low levels in stages V–IX and 5-fold higher levels in stages XIII, XIV, and I. This result correlated very well to recent data from other laboratories which used the dissection method to determine other parameters of FSH action. Altogether these studies suggest that the primary action of FSH in the adult male rat is cyclic in nature and may be confined to stages XII–IV.

We examined the possible regulation of steady state levels of FSHR mRNA by hormones and growth factors that play important roles in spermatogenesis. We examined whether or not FSH or phorbol ester could regulate the FSHR mRNA level. Cultured Sertoli cells from 20-day-old rats were treated with different combinations of hormones, phorbol esters and vitamin A. The amount of FSHR mRNA in these cells was then assayed by Northern blots. The data was normalized to the mRNA levels of actin and clusterin which have been shown to be unchanged in cell culture in the presence of different hormones. We found that after 3 days in culture, FSH, insulin, retinol or testosterone added individually did not alter FSHR mRNA levels (relative to total poly A^+ mRNA). However, addition of all 4 reagents resulted in a 1.5–2-fold increase in the steady state FSHR mRNA levels. Studies from another laboratory have shown that FSH added to cultured Sertoli cells down regulated the level of FSHR mRNA within 4 h of addition of hormone to cultured Sertoli cells [21]. Transcriptional run-on experiments by these same investigators showed that FSH did not inhibit the initiation of transcription of the FSHR gene and that by 16 h after addition of hormone the FSHR mRNA levels had returned to normal. The authors suggest that these results show that the FSH induced receptor down regulation of FSHR mRNA occurred through a post-transcriptional mechanism.

A report which showed that phorbol esters desensitize Sertoli cells to FSH prompted the examination of the effect of phorbol esters on the steady state levels of FSHR mRNA [22]. The addition of phorbol esters to cultured Sertoli cells results in a rapid and dramatic decrease in FSHR mRNA followed by return to steady state levels [14]. The mechanism of this inhibitory action is unknown (transcriptional or post-transcriptional) but the results clearly show that phorbol esters can alter the FSH response of Sertoli cells.

5′ FLANKING REGION OF FSHR GENE

Understanding the regulation of the FSHR mRNA at the transcriptional level involves an analysis of the

promoter regions associated with the gene. We have characterized the 5′-flanking region of the rat FSHR gene [12]. Primer extension and S1 nuclease experiments revealed the presence of two major transcriptional start sites at positions −80 and −98 relative to the translational start site. The promoter region immediately upstream from the transcriptional start site did not contain conventional TATA or CCAAT elements. The promoter region has characteristics which are similar to promoters of genes coding for "housekeeping" functions [23]. Characteristics of these promoters include multiple transcription initiation sites, absence of TATA and CCAAT boxes, and high GC content. The 5′-flanking regions of the LH receptor gene and the TSH receptor gene have also been sequenced and show similar characteristics. The sequences of these promoter regions have a higher overall GC content than does the corresponding region of the FSH receptor gene [24–26].

This promoter is of considerable interest since it should contain cell specific enhancer elements which may direct expression of trans genes in Sertoli cells or granulosa cells. In order to analyze the promoter we constructed several fusion genes containing from 280 to 5 kb of DNA 5′ to the translational start site linked to the reporter gene, chloramphenicol acetyltransferase. These gene constructs were transfected into cultured Sertoli cells, COS-7 cells and a murine cell line derived from Sertoli cells designated as MSC-1. All of these constructs actively promoted transcription of the reporter gene in primary cultures of Sertoli cells, NIH 3T3 cells, MA-10 cells (a Leydig cell line) and MSC-1 cells but not in COS-7 cells. These results showed that the DNA elements responsible for transcription reside in the first 280 bp of the 5′-flanking region and that promoter activity was not cell specific in transient transfection assays. However, when the 5 kb piece of promoter was used to drive the transcription of β-galactosidase in transgenic animals, it was found that the promoter directed expression of the gene only into testes or ovaries of transgenic mice. Thus, even though the promoter is promiscuous in transient transfections it works in a very cell specific mode in transgenic mice.

The apparent discrepancy in the results of the transient transfection assays and the results from the transgenic mice have a number of possible explanations. One possibility is that in the transgenic mice the transgene is inactivated by methylation in normally non-expressing tissues. We have examined the methylation state of the endogenous FSHR gene in Sertoli cells and in other non-expressing tissues. We examined a specific CCGG sequence in the promoter and found it to be non-methylated in Sertoli cells of the male and to be methylated in all other tissues we examined [27].

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