

The Follicle-Stimulating Hormone Receptor: Biochemistry, Molecular Biology, Physiology, and Pathophysiology*

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I. Introduction

FSH IS the central hormone of mammalian reproduction, necessary for gonadal development and maturation at puberty and for gamete production during the fertile phase of life (1, 2). Together with LH, this gonadotropin is produced and secreted by the pituitary gland as a highly heterogeneous glycoprotein (3, 4). FSH acts by binding to specific receptors, localized exclusively in the gonads. The FSH receptor belongs to the family of G protein-coupled receptors, complex transmembrane proteins characterized by seven hydrophobic helices inserted in the plasmalemma and by intracellular and extracellular domains of variable dimensions depending on the type of ligand (5). The intracellular portion of the FSH receptor is coupled to a G_s protein and, upon receptor activation by the hormonal interaction with the extracellular domain, initiates the cascade of events that ultimately leads to the specific biological effects of the gonadotropin.

After two decades of investigations using classic biochemical approaches, the FSH receptor cDNA was finally cloned in 1990, the last in the group of closely related receptors for the glycoprotein hormones (6). Thereafter the first mutations were described, with impressive impact on the reproductive phenotype (7, 8). The new knowledge emerging from naturally occurring mutations and from *in vitro* molecular work provides important new insights into FSH physiology and pathophysiology. Unlike the cognate LH and TSH receptor, the functional properties of which have been recently reviewed by several authors (9–13), the large body of FSH receptor research has not yet been comprehensively considered. Feeling the need for an integrated view of the relevant knowledge about biochemical and molecular properties of the FSH receptor at this stage, we compiled this article with the aim of providing both a state-of-the-art review and a stimulating springboard for further pertinent research.

II. Biochemical Properties of the FSH Receptor: A Historical Prelude

The first experimental evidence that gonads possess binding sites specific for FSH dates back to the beginning of the early 1970s (14–15). The earliest study, performed on rat testicular mince using tritiated human FSH, in fact showed the salient characteristics of the FSH receptor. The hormonal binding was rapid, specific, saturable, and temperature-dependent. FSH-binding sites were found only in the seminif-

erous epithelium, associated with the cell membrane, and were proteinaceous in nature. Finally, the rat FSH receptor did not exhibit species specificity and was able to bind FSH from different species (14). In the following years, several groups attempted to isolate and characterize FSH receptors. These classic experiments still represent a major contribution to FSH receptor research, having set important milestones of reproductive physiology that cannot be overshadowed by today's molecular research (Table 1).

The original work directed toward isolating and purifying the FSH receptor was based almost exclusively on testis material. Most studies on the characterization of FSH-binding sites in the premolecular era were performed on murine (14, 16–20) and bovine FSH receptors (21–28), but FSH-receptor interactions were also studied in the swine (29), in nonhuman primates (30–32), and in humans (20, 33–36). The FSH receptor of other animal species was investigated only later using molecular biology techniques.

After the initial attempts to isolate the rat and calf FSH receptor (17, 18, 22, 37), two classes of FSH-binding sites were detected, with high affinity and low capacity and with low affinity and high capacity, respectively (14, 16, 17, 37, 38). The low-affinity component, however, turned out to be artifactual (38), and only one class of high-affinity binding sites can be demonstrated in purified preparations (22). The specific binding of FSH is rapid at physiological temperatures and reaches saturation within 4 h in all systems investigated (14, 16, 17, 21, 22, 29–31, 39).

FSH receptors are particularly abundant in the immature bovine testis, and the receptor concentration is higher in the bovine calf compared with the mature rat and human testis (22, 29, 40). The testicular content of FSH-binding sites increases with age in the rat and the bovine, where the appearance of FSH receptors precedes the increase in plasma FSH, testicular growth, and the increase of LH receptor concentration at puberty (41). There is very little, if any, species specificity in the FSH-receptor interaction, which shows sim-

ilar characteristics in homologous as well as in heterologous systems (32, 37).

Concerning the precise localization of the FSH receptor, autoradiographic studies showed that labeled FSH was selectively localized on the surface of the Sertoli cells, outside the tight junctions (19). After hyperosmotic fixation, which produces preferential shrinkage of the cells of the basal compartment, FSH-binding sites were also evident on spermatogonia (42), but the spermatogonial localization was never confirmed (43). A recent immunocytochemical study with an antibody directed against the human FSH receptor showed uniform labeling of granulosa cell membranes and of the basal pole of Sertoli cells around the spermatogonia (44). Interstitial macrophages were shown to bind and accumulate FSH (19, 33) and to respond to FSH administration (45–47). The endothelial cells of the small vessels in the interstitial space were also stained (44). Whether these localizations are related to the transport of the large glycoprotein hormone to the target site remains to be determined (48–50).

Earlier experiments showed that the FSH receptor is a glycoprotein (17, 21, 30) and that FSH-receptor interaction is partially dependent on the presence of phospholipids when membrane preparations are used (17, 22, 30, 51). In fact, G protein-coupled receptors are anchored to plasma membranes by fatty acylation or protein lipidation, which stabilizes protein conformation and possibly plays some role in signal transduction (52, 53). Moreover, FSH receptor binding is dependent on the integrity of disulfide bonds (17, 30), which stabilize the receptor conformation, whereas a postulated role in maintaining a subunit structure (54) has not been confirmed.

The purification studies yielded widely variable models of the size and structure of the receptor (22, 24, 28, 34, 54–56). Even after the cloned cDNA predicted a single peptide chain with a molecular mass of 75 kDa (6), the mature FSH receptor from rat Sertoli cell membranes is occasionally found to have

TABLE 1. Chronology of FSH receptor research before cloning of FSH receptor cDNA

| Year | Finding/investigation | Reference |
|-----------|--|-------------|
| 1972 | Specific binding of FSH demonstrated in rat testis mince | 14 |
| 1974 | Characterization of the FSH-receptor interactions in rat testis homogenates and development of the first FSH radioligand receptor assay | 16 |
| 1975 | Characterization of the FSH-receptor interactions in calf testis membranes | 21 |
| | Identification of the calf testis as the richest source of FSH binding sites and development of a calf testis-based FSH radioligand receptor assay | 40 |
| 1976 | Purification of rat FSH receptor in sucrose gradient | 17 |
| 1977 | Solubilization of calf FSH receptor | 22 |
| | Solubilization of rat FSH receptor | 18 |
| | Autoradiographic localization of FSH binding sites in the testis | 19 |
| 1978 | Characterization of FSH-receptor interactions in porcine testis | 29 |
| | Assessment of the developmental regulation of FSH receptor | 41 |
| 1978–1982 | Studies on FSH-induced down regulation of FSH receptor and postreceptor events | 59–63 |
| 1978–1987 | Localization and properties of the FSH receptor in normal and pathologic human testis | 20,21,34–36 |
| 1982–1984 | Characterization of the FSH receptor in monkey testis | 30–32 |
| 1983 | Immunocytochemical localization of FSH binding sites in rat and human testis | 20 |
| 1985–1986 | Assessment of the quaternary structure for the FSH receptor | 23,24,54 |
| 1986 | Stable solubilization of calf FSH receptors without the aid of stabilizing agents | 27 |
| 1987 | Demonstration of physical and functional association of the FSH receptor to G _s protein | 25 |
| 1988 | Reconstitution of the solubilized calf FSH receptor into proteoliposomes retaining G _s and adenylate cyclase association | 26 |
| 1990 | Purification of the solubilized calf FSH receptor, confirming its oligomeric nature | 28 |
| | Cloning of the rat FSH receptor cDNA | 6 |

a much larger size, and the controversy about the receptor structure is still not definitely resolved (57).

The involvement of guanine nucleotides in FSH receptor function had already become evident at a time when G proteins were not yet known (17), and the stable solubilization of the bovine FSH receptor demonstrated its physical and functional coupling to G_s protein (26, 27). While indirect effects on the phosphoinositide pathway are possible (54), the FSH-dependent increase in intracellular cAMP was soon recognized to be the main signal transduction mechanism in Sertoli and granulosa cells (58) and FSH induces receptor down-regulation (59–63). The most recent knowledge about receptor coupling, signal transduction, and receptor desensitization derives, however, from studies with recombinant receptors transfected in cell lines and will be reviewed in Section VII.

III. Molecular Structure of the FSH Receptor

A. Cloning of the FSH receptor

The use of recombinant DNA technology represented a major breakthrough in the structure of the FSH receptor. The first sequences of a putative FSH receptor DNA fragment were reported in 1989 by Vassart's group (64) who discovered them while cloning the human TSH receptor. Based on conserved regions in the transmembrane segments II to VII of G protein-coupled receptors, degenerate oligonucleotide primers were designed, and subsequent PCR amplification led to the isolation of one genomic clone that yielded the partial amino acid (aa) sequence of a special subfamily member of G protein-coupled receptors. In particular, the sequence did not contain the canonical Asp-Arg-Tyr triplet motif, present in a whole variety of receptors belonging to this family. Using this clone as a probe in Northern blot hybridization, a prominent 2.6-kb transcript was detected in the ovary and the testis, whereas other tissues were negative. This DNA fragment encoding part of the transmembrane domain was designated to be part of the human FSH receptor and was used further under low-stringency hybridization conditions to isolate the human TSH receptor.

In 1989 the rat LH receptor was cloned by Seeburg's and Segaloff's groups using a classic molecular biology approach for the isolation of target cDNA as follows (65). First, Segaloff's group purified rat LH receptor protein by affinity chromatography. Based on the aa sequence of the N-terminal side, degenerate oligonucleotide primers allowed the PCR amplification of a specific cDNA product, which was then used to isolate the corresponding full-length cDNA from an ovarian cDNA library. Sequence analysis of the cDNA revealed that the rat LH receptor is a single potentially glycosylated protein containing an unusually large, predicted extracellular domain.

The similarity of the cloned LH receptor and TSH receptor and the observation that all three glycoprotein hormones act on their respective receptors via the cAMP pathway led to the assumption that the structural design of the FSH receptor should display similar characteristics. Since Sertoli cells are the sole specific target of FSH action and do not bind LH, cDNA probes corresponding to selected regions of the LH

receptor were used to screen a rat Sertoli cell cDNA library. The isolated cDNA had an approximate size of 2.3 kb, and its specificity was investigated by functional expression studies. Human embryonic kidney cells transfected with the putative receptor cDNA displayed an FSH-dependent and saturable increase in intracellular cAMP. In contrast, no cAMP stimulation was observed when using human (h) CG or hTSH, indicating the successful cloning of the rat FSH receptor (6) (Fig. 1).

This first description of the rat FSH receptor-cloning procedure enabled other investigators to identify and characterize FSH receptors from different species. To date, the sequences of the human (66–68), monkey (69), equine (70), ovine (71), pig (72), bovine (73), chicken (74), and reptile (75) FSH receptor are known. The strategies used to obtain the cDNA range from RT-PCR using primers based on sequence homology within the different species, to the isolation of clones from cDNA libraries constructed from ovarian or testicular tissue using FSH receptor cDNAs from other species as a probe. Analysis of the cDNAs revealed a 2085-nucleotide open reading frame (ORF) in most of the species, 2076 nucleotides in the rat (6), and 2082 nucleotides in the equine receptor (70). The translation initiation codon is preceded by one or several stop codons and thereby the nucleotide sequence between position –3 and +4 (defining the first nucleotide of the translational codon ATG as +1) does not correspond to a perfect consensus sequence for the initiation of translation according to Kozak (76). The untranslated 3'-end of the cDNA contains one or two putative polyadenylation signals.

Several sequences of the human FSH receptor have been reported, differing at several nucleotide positions and resulting, in some cases, in aa substitutions (77–79). Some of these discrepancies were revised (77) but others, such as the presence of amino acid Thr or Ala at position 307 and Ser or Asn at position 680, are not due to inaccurate sequencing. Since the different cDNA sequences were generated from testicular (67, 78) or ovarian tissue (66), it was originally postulated that these differences might be related to sex-specific changes. It is now clear that the observed substitu-

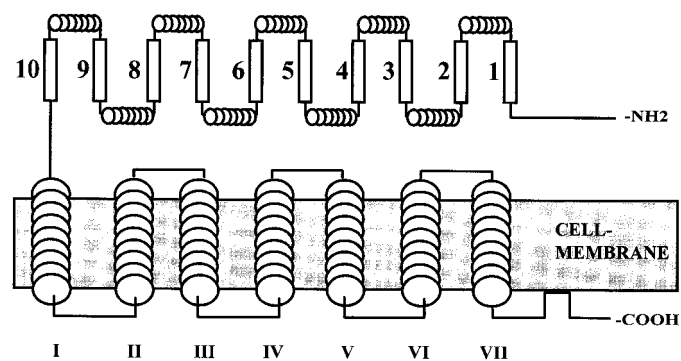


FIG. 1. Schematic model of the FSH receptor. The extracellular domain consists of several LRRs that are made up of alternating β -sheets (indicated by rectangles numbered consecutively in Arabic numerals according to the alignment shown in Fig. 3) and α -helices (indicated by the coils). The transmembrane domain consists of seven hydrophobic segments (Roman numerals) spanning the cell membrane and connected by intra- and extracellular loops. Within the intracellular domain a putative fourth intracellular loop is depicted.

tions in the FSH receptor cDNA are due to two polymorphic sites in the FSH receptor gene (see *Section X*).

B. Predicted primary structure of the FSH receptor

The predicted human FSH receptor protein (66–68) is composed of 695 aa (692 aa in the rat and 691 aa in the equine), including the first 17 aa, which encode a hydrophobic signal peptide (Fig. 2: aa numbering maintained throughout this article). Therefore, the mature protein is likely to consist of 678 aa (675 aa in the rat and 677 in the equine). Depending on the species, the calculated molecular mass based on the cDNA sequence for the mature receptor protein ranges between 75 and 76.5 kDa. Further characterization of the aa sequence and hydropathy plot analysis revealed that the FSH receptor consists of a huge hydrophilic domain followed by hydrophobic segments spanning the membrane seven times, with a length of 21–24 aa. At the C terminus the sequence predicts a highly basic cytosolic segment.

The extracellular domain of the receptor is composed of 349 aa [348 for the equine (70) and the rat (6)], followed by 264 aa encoding the transmembrane domain. The relatively short carboxy-terminal intracellular domain consists of 65 aa (63 in the rat). The homology between different mammalian species is generally high and reaches 90% in the transmembrane domain. Although the overall interspecies homology of the extracellular domain is about 85%, it is in this portion that the most variable region can be identified, in a segment just before the first transmembrane domain. Other stretches of pronounced aa differences are located in the C-terminal tail, displaying an overall between-species homology of 80%. Compared with the LH receptor, the homology is high in the transmembrane domain (70%), whereas it drops to 42% in the extracellular domain and to 48% in the intracellular domain, respectively. A similar pattern of homology is observed when the FSH receptor is compared with the TSH receptor (Fig. 2).

1. Extracellular domain. The extracellular domain of the FSH receptor displays several significant primary and secondary structure features. It is composed of several imperfectly replicated units of approximately 24 residues each. This characteristic motif is also present in the LH and in the TSH receptors and, in part, even in the so-called remainder forms of the recently cloned glycoprotein hormone receptor ancestor (80–82). Similar motifs, termed leucine-rich repeats (LRR), are found in proteins involved in cell-specific adhesion and protein-protein interaction in species extending from yeast to man (Refs. 65 and 83 and references therein). The crystal structure of the porcine ribonuclease inhibitor, containing LRR, has been resolved recently (83). The individual repeats constitute structural units of alternating β -sheets and α -helices, probably occurring in the gonadotropin receptors as well. The nonglobular shape of the structure and the exposed face of the parallel β -sheet may explain the involvement of LRR in strong protein-protein interactions (83) (Fig. 1).

Alignment of exons 2–9 (see also *Section IV*) in the extracellular domain of the FSH receptor reveals at least 10 imperfect LRR motifs (Fig. 3). The conserved positions are

occupied by Ile, Leu, Val, Ala, and Phe, aa belonging to the aliphatic group. The LRR pattern is highly conserved in exons 2–8, less so in exon 9. Exon 1 and the C-terminal part of the extracellular domain of the FSH receptor, encoded by exon 10, do not conform to the consensus motif. The consensus sequence of the LRR in the FSH receptor is homologous to the motif found in the LH receptor (83) and in the TSH receptor (12). Due to their amphiphatic nature, the repeats might confer the dual property of interacting both with the hormone and the transmembrane domain (84). Within the FSH receptor, repeats 1–10 participate in FSH binding (85), and the binding specificity is probably localized between LRR 5 and LRR 10 (see *Section VII.B*) (86).

The extracellular domain of the FSH receptor contains several cysteine residues located primarily in exons 1 and 10, two of which are in adjacent position. Eight cysteine residues are perfectly conserved in the LH and TSH receptor, suggesting a crucial role for the conformational integrity of the large extracellular domain of all the glycoprotein hormone receptors. The extracellular domain of the FSH receptor has three potential sites for N-linked glycosylation conserved in every species, at positions 191, 199, and 293, respectively. In the human and monkey FSH receptor, a fourth potential site can be allocated to position 318, and in the equine receptor an additional site can be found at position 268. The glycosylation site at position 191 is highly conserved among the LH, TSH, and FSH receptors (Fig. 2), whereas the others cannot be aligned with the remaining six potential glycosylation sites of the LH receptor and five potential glycosylation sites of the TSH receptor. Although it was suggested that the glycosylation pattern of the FSH receptor might affect hormone binding, recent studies indicate that glycosylation is rather required for proper folding of the receptor protein and trafficking to the membrane (see *Section VIII*) (85).

2. Transmembrane domain. The structural motif of the heptahelical or serpentine transmembrane domain is typical of members belonging to the superfamily of G protein-coupled receptors (87, 88) (Fig. 1). In each member of this group the motif is characterized by seven hydrophobic stretches of 20–25 aa predicted to form transmembrane α -helices, connected by alternating extracellular and intracellular loops. Similar to members of this receptor family, the FSH receptor contains two highly conserved Cys residues (positions 442 and 517) in the first and second extracellular loop, predicted to form an intramolecular disulfide bridge (5), which constrains the conformation of the protein (6). The highly conserved Asp-Arg-Tyr triplet motif (5), believed to play a central role in the interaction between receptor and G protein, is present in the FSH receptor in a modified version in which Asp is replaced by Glu (positions 466–468). The same substitution is observed in the corresponding triplet motif of the LH and TSH receptor.

Comparison of the transmembrane domains of the FSH, LH, and TSH receptor reveals that transmembrane domains 2, 3, and 4 are highly conserved, whereas conservation in the other four transmembrane regions is lower. Proline residues, which may be necessary for proper insertion of the protein into the membrane, are homologous in the fourth, sixth, and

| | | |
|------------|--|-----|
| human FSHR | M-----ALLLVSLLAFLSLGSGCHHRICHCSNRVFLCQESKVTETIPSDL | 44 |
| human LHR | MKQ-RFSALQLKLLLLQLPFLPRALREALCEPFCNCPVDPGGLRCFGPTA | 49 |
| human TSHR | MRPADLLQLVLLDLPRLDGLGMCSSPPCECHQEEDFRVTKCKDIQRIPSL | 50 |
| human FSHR | PRNAIELRFVLTCLRVIQKGAFGSGFDLEKIEISQNDVLEVEADVFSNL | 94 |
| human LHR | GLTRLSLAYL--PVKVIPSQAIFRGLNEVVKIEISQIDSLERIEANAFDNL | 97 |
| human TSHR | FPSTQTLKLIETHLRITIPSHAFSNLNPISRIYVSIDVTLQQLESHSFYNL | 100 |
| human FSHR | PKLHEIRIEKANNLLYINPEAFQNLNLYLLISNTGIKHLDPDVHKIHSL | 144 |
| human LHR | LNLSEILIQNTKNLRYIEPGAFINLPRKYLSCINTGIRKFPDVTKVFS | 147 |
| human TSHR | SKVTHIEIRNTRNLTYIDPDALKEPLKFLGFNTGLKMFDDLTKVYST | 150 |
| human FSHR | Q-KVLLDIQDNINIHTIERNISFVGLSFESVILWLNKNGIQEIHNCANFGT | 193 |
| human LHR | ESNFILEICDNLHITTPGNAFQGMNNEVTLKLYGNFEEVQMHAFNGT | 197 |
| human TSHR | DIFFILEITDNPYMTSIPVNAFQGLCNETLTLKLYNNGFTSVQGYAFNGT | 200 |
| human FSHR | QLDELNLSDNNNLEELPNDVFHGA-SGPVILDISRTRISLPSYGLENLK | 242 |
| human LHR | TLTSLLEKENVHLEKMHNGAFRGA-TGPKTLDISSTKQLPFSYGLESYQ | 246 |
| human TSHR | KLDAVYLNKNKYLTVIDKDAFGGVYSGPSLLDVSQTSVTALPSKGLEHLK | 250 |
| human FSHR | KLRARSTYNLKKLPTLEKLVALMEASLTPSHCCAFANWRRQISELHPIC | 292 |
| human LHR | RLIATSSYSLKKLPSRETFFVNLEATLTPSHCCAFRNLPKQNFSSHIS | 296 |
| human TSHR | ELIARNTWTLKKLPLSLFLHLTRADLSYPSHCCAFKNQKIRGILESML | 300 |
| human FSHR | NKSILRQEVDMYMTQTRGQRSSLAEDNESSYSRG----- | 325 |
| human LHR | SENFQKQCESTVRKVSNTKLYSSMLAES----- | 324 |
| human TSHR | CNESSMQSLRQRKSVNALNSPLHQEYEENLGDSIVGYKEKSKFQDTHNNA | 350 |
| human FSHR | -----FDMTYTEFDYDLCEVVDVTCSP | 348 |
| human LHR | -----ELSGWDYEGYGFCLPKTP-RCAP | 345 |
| human TSHR | HYYVFEEQEDEIIGFQELKNPQEEITLQAFDSHYDYITICGDSMDVCTP | 400 |
| human FSHR | KPDAFNPCEDIMGYNLRVLIWFISILAITGNIIVLVILTTSQYKLTVPR | 398 |
| human LHR | EPDAFNPCEDIMGYDFLRVLIWLNILAIMGNMIVLVLLTSRYKLTVPR | 395 |
| human TSHR | KSDEFNPCEDIMGYKFLRVVWFVSLALLGNVFLILLTSYKLNVR | 450 |
| human FSHR | FLMCNLAFLADLCIGIYLLLIASVDIHTKSQYHNYAIDWQTGAGCPAGGF | 448 |
| human LHR | FLMCNLSFADFCMGLYLLLIASVDSQTKGQYHNAIDWQTGSGCSPAGFF | 445 |
| human TSHR | FLMCNLAFLADFCMGMYLIIASVDLYTHSEYHNAIDWQTGPGCNAGGF | 500 |
| human FSHR | TVFASELSVYTIITAITLERWHTITHAMQLDCKVQLRHASVMVMGWIFAF | 498 |
| human LHR | TVFASELSVYTIITVITLERWHTITYAIHLQKLRRLRAILIMLGGLFSS | 495 |
| human TSHR | TVFASELSVYTIITVITLERWYAITFAMRLDRKIRLRHACAIMVGGWVCCF | 550 |
| human FSHR | AAALFPIFGISSYMKVSI CLEPMDIDSPLSQLYVMSLLVLNVLAFVVICGQ | 548 |
| human LHR | LIAMLPVGVSNYMKVSI CLEPMDVETLTSQYIILTLILNVAFFIICAC | 545 |
| human TSHR | LLALLPLVGISSYAKVSI CLEPMDTETPLALAYIVFVLTLLNIVAFVIVCC | 600 |
| human FSHR | YIHIYLTVRNPNIVSSSDTRIAKRMAMLIFTDFLCMAPISEFFAISASLK | 598 |
| human LHR | YIKIYFAVRNPELMATNKDKTKIAKRMALIFTDFTCMAPISEFFAISAAFK | 595 |
| human TSHR | HVKIYITVRNPQYNPGDKDKTKIAKRMALIFTDFICMAPISEFFALSAILN | 650 |
| human FSHR | VPLITVSKAKILLVLFHPINSCANPFLYAIFTKNFRDRDFILLSKGCYIE | 648 |
| human LHR | VPLITVTSKAVLLVLFYFPLINSCANPFLYAIFTKTFQRDFILLSKFGCCK | 645 |
| human TSHR | KPLITVSNKAILLVLFYPLINSCANPFLYAIFTKAFQRDVILLSKFGICK | 700 |
| human FSHR | MQAQIYR-----TETSSTVHNTHPRNGHCSSAPR----VTSGSTYILV | 687 |
| human LHR | RRAELYRRKDFSAYTSNCKNGFTGSNKPQSSTKLKLS----TLHCQGTALL | 691 |
| human TSHR | RQAQAYRQQRVPPKNSTDIQVQKVTHDMRQGLHNMEDVYELIENSHLTPK | 750 |
| human FSHR | PLSHLAQN | 695 |
| human LHR | DKTRYTEC | 699 |
| human TSHR | KQQQISEE | 758 |

FIG. 2. Amino acid sequence of the human FSH receptor. Amino acid numbering includes the 17-residue signal peptide here and throughout the paper. For comparison the aa sequences of the human LH receptor and TSH receptor are shown below. Identical amino acids are indicated by dots. The seven-membrane spanning domains are boxed and designated TM 1–TM 7.

seventh transmembrane segment of all glycoprotein hormone receptors. Between the cytoplasmic loops the highest aa homology can be noted in the first loop. The third cytoplasmic loop is significantly shorter compared with other members of the G protein-coupled receptor family, and the

homology between the FSH, LH, and TSH receptor in this region is low.

3. C-terminal domain (aa 631–695). The intracellular domain of the glycoprotein hormone receptors displays some homol-

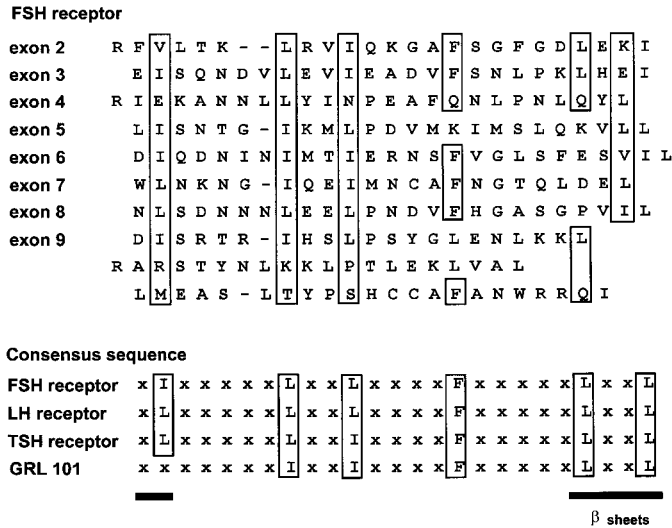


FIG. 3. *Upper*, Alignment of the aa sequences of exons 2–9 of the human FSH receptor gene. Aliphatic aa that conform to the repeat pattern of the LRR proteins are boxed. Exon 1 and the extracellular part of exon 10 did not display any consensus motif and were therefore excluded. Exon 9, consisting of 63 aa compared with 23–24 aa for exons 2–8, was divided into three parts according to the highest homology with the LRR pattern. *Lower*, Consensus sequences for LRRs observed in the FSH, LH, and TSH receptors. Furthermore, the LRR consensus sequence of a G protein-coupled receptor from the mollusc *Lymnea stagnalis* is given, indicating the highly conserved pattern of these motifs early in evolution. *Black bars* indicate the β -sheet formation of the LRRs.

ogy only in the N-terminal portion (aa 631–659 of the FSH receptor). Moreover, within different species the C-terminal domain of the FSH receptor is quite heterogeneous. For example, there is a gap of two aa in the rat FSH receptor. The domain is rich in serine and threonine residues, which are potential phosphorylation sites (see *Section VIII*), but a typical consensus recognition site for the cAMP-dependent kinase (Arg-Arg-X-Ser/Thr) is lacking. The cysteine residues might be palmitoylated, thereby serving as an additional membrane anchor of the cytoplasmic receptor tail. This fourth intracellular loop might be relevant for receptor coupling and regulation of signal transduction. Two of these cysteine residues (positions 646 and 672) are conserved within the different species, whereas the human and equine FSH receptors possess an additional cysteine, at position 644 in the human receptor and at position 671 in the equine receptor, respectively.

C. Molecular mass of the FSH receptor

The predicted glycoprotein nature of the FSH receptor, with the inherent possibility of glycosylation variants, might be responsible for the differences in its molecular mass reported in the literature. Based on the ORF of the cDNA, a molecular mass of approximately 75 kDa can be calculated. However, several groups have demonstrated precursors of the glycosylated protein or isoforms of the mature receptor with lower or higher molecular mass (44, 57, 89, 90).

Taken together, the reports on mass determination indicate that the mature, glycosylated, recombinant FSH receptor is a protein of approximately 80 kDa (78, 85). Furthermore,

binding of FSH does not require prior receptor oligomerization, although aggregation might be necessary for stability and/or signal transduction. Cleavage of the related LH and TSH receptor proteins, resulting in lower molecular mass forms, has been described (11, 91). The FSH receptor sequence displays five putative dibasic cleavage sites at positions 242, 253, 282, 572, and 634, respectively, conserved within the different species. Some of them are also found in the LH and in the TSH receptor at corresponding sites (Fig. 2). To date, however, the studies on tissues naturally expressing the FSH receptor did not reveal any evidence for spontaneous cleavage of the FSH receptor protein (44).

IV. The FSH Receptor Gene

A. Chromosomal localization

The chromosomal mapping of the FSH receptor gene has been performed by fluorescence *in situ* hybridization using cDNA or genomic probes and by linkage analysis (7, 92–94). The FSH receptor gene is mapped to chromosome 2 p21 in the human (92, 93) and to chromosome 3 in the sheep and the pig (72, 94). Interestingly, the LH receptor gene can be mapped to the same chromosomal location in the human and in the sheep (94, 95). Pulse field analysis to determine the physical distance between the two genes in the human revealed no common band within 1100 kbp, suggesting that they might be located at some distance from each other (92). The related human TSH receptor is located on chromosome 14 q31 (96).

The large extracellular domain of the glycoprotein hormone receptors is a unique feature within the G protein-coupled receptor family. The similar genomic arrangement and the nearly identical exon/intron boundaries of the three receptor genes, together with the proximity of the two gonadotropin receptor genes, indicate a common ancestor. This ancestral gene could have evolved first by chromosomal duplication, followed by duplication of the gene (94). Recent studies on genome evolution have shown that human FSH, LH, and TSH receptors are located in a group of chromosomes with extensive paralogous connections, *i.e.*, containing genes arising from gene duplication and subsequent divergence (97). A detailed aa sequence analysis of the three glycoprotein hormone receptors reveals a closer sequence similarity between the FSH and LH receptor than between either to the TSH receptor. This finding and the lack of a duplication locus for the TSH receptor have led to the hypothesis that the LH receptor and TSH receptor have evolved by chromosomal duplication. Further duplication of the FSH receptor/LH receptor locus and subsequent functional divergence would then have resulted in the two gonadotropin receptors present in mammals today (94).

Further evolutionary insights have recently been obtained from the identification of glycoprotein hormone receptor-like receptors in invertebrates. In the mollusc *Lymnea stagnalis*, a G protein-coupled receptor with a very large extracellular domain has been recently cloned (81). The N-terminal portion of the extracellular domain consists of several Cys-containing repeats, a motif present also in low-density lipoprotein receptors, whereas the second part of the

extracellular domain contains six LRR. Thus, this receptor, in a phylogenetically very old species, might be a remainder of the common ancestor from which the genes encoding the mammalian glycoprotein hormone receptors have evolved through duplication of the LRR and removal of the Cys-containing repeats. Another G protein-coupled receptor, recently cloned from sea anemones, displays striking similarity to the glycoprotein hormone receptors. In fact, it possesses a huge extracellular domain, shows alternative splicing of the primary transcript, and encloses two introns with position and intron phase identical to those of introns 7 and 8 of the glycoprotein hormone receptor genes (80). These characteristic similarities have been shown also in a G protein-coupled receptor cloned from *Drosophila melanogaster*, which appears to be involved in developmental processes of insects (82). These invertebrate receptors might reflect steps in the evolutionary process of defining and remodeling the structure of glycoprotein hormone receptors.

B. Structure and organization of the FSH receptor gene

The structure and organization of the FSH receptor gene have been investigated in humans and rats (98, 99). The FSH receptor gene is a single-copy gene and spans a region of 54 kbp in the human and 84 kbp in the rat, as judged from restriction analysis of genomic clones and size determination of PCR products. It consists of 10 exons and nine introns (Fig. 4). The extracellular domain of the human receptor is encoded by nine exons ranging from 69–251 bp. The C-terminal part of the extracellular domain, transmembrane and the intracellular domain, is encoded by exon 10 with more than 1234 bp (99). Overall, the human gene encodes 695 aa, including a signal peptide with 17 aa. The nine introns vary greatly in their corresponding sizes from 108 bp for intron 7 to 15 kbp for intron 1. The exon-intron boundaries correspond to a canonical splice consensus sequence conserved in all exons. The introns are in phase 2, and the aa that resides at nearly each exon/intron junction is either Leu or Ile.

The structure and organization of the human and rat FSH receptor gene display striking similarities. The exon sizes are identical, except for exon 10, where an extension by nine bases can be found in the human receptor, corresponding to three aa at position 316 and position 381–382. At the nucleotide level the homology is above 80%, and at the aa level it even reaches 100% in the case of exon 3. The exon/intron junctions are identical in both species (98, 99).

With 70 kbp for the LH receptor (100), 60 kbp for the TSH receptor (101), and 54 kbp for the FSH receptor, the three human glycoprotein hormone receptor genes are huge. The FSH and TSH receptors consist of 10 exons, while the LH receptor has 11 exons. The similarity between the genes is high. The sizes of several exons of the extracellular domain are identical in the three genes. The other exons differ only by three bases; the exception is the additional exon 10 of the LH receptor, which is unique as it contains three putative N-linked glycosylation sites (102). Furthermore, the intronic sequences of the 5'-end of exon 11 of the LH receptor correspond to promoter and regulatory regions of the intronless genes of other G protein-coupled receptors (103, 104). Probably this last intron was lost during the evolution of the

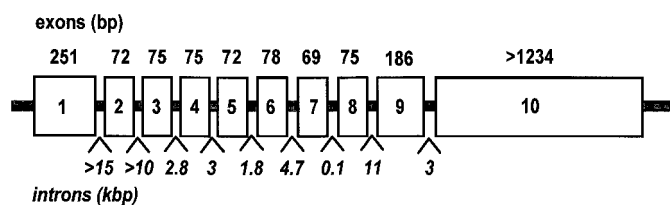


FIG. 4. Structural organization of the human FSH receptor gene. The boxes 1–10 indicate the different exons of the FSH receptor gene. Exons 1–9 encode the extracellular domain, whereas exon 10 encodes the C-terminal part of the extracellular domain, the transmembrane domain, and the intracellular domain. The number of base pairs is given above each exon. The intron length between the different exons is given below.

cognate FSH and TSH receptor, resulting in the structural arrangement of ten exons and nine introns.

C. The promoter of the FSH receptor gene

The FSH receptor gene expression is highly tissue-specific and strictly dependent upon different hormonal stimulation. To elucidate the regulatory mechanisms of the expression, several groups of investigators tried to characterize the promoter response elements and corresponding factors in the 5'-flanking region of the FSH receptor gene. The putative promoter regions of the human, rat, and mouse FSH receptor have been cloned, the sequences were analyzed, and different promoter constructs were investigated in functional studies using different cell types (98, 105–107). Using ribonuclease (RNase) protection assay or primer extension analysis, a major transcriptional start site has been located at position –99 in the human (104) and at –534 in the mouse (107) receptor, relative to the translational start site. In the rat, two major transcriptional start sites at positions –80 and –98 were found (98). In all species, additional, less marked transcriptional initiation sites have been observed. The transcriptional start sites of the human FSH receptor mRNA are identical in the testis and ovary, thereby excluding the possibility that the sex-specific regulation of gene expression makes use of different transcriptional start sites (106).

The 5'-flanking regions of the different genes lack canonical TATA or CCAAT promoter elements. Furthermore, GC box motifs, binding sites for the promoter-specific transcription factor SP-1, are present in the LH receptor (100, 104) but not in the FSH receptor promoter. Searching for further transcription binding sites revealed an activator protein 1-binding site at position –214 in the rat FSH receptor gene (98). Although the treatment of cultured rat Sertoli cells with phorbol esters results in a decreased response of the cells to FSH (108), the importance of this activator protein 1-binding site is questionable since it is not present in the human or mouse promoter. Similarly, a consensus estrogen-responsive element found in the human promoter at positions –217 to –221 has no correspondent in the rat or mouse promoter (106). A described initiator region (InR), encompassing a transcriptional start site, is conserved only in mouse and rat, whereas an E box element, interacting with a family of basic helix-loop-helix transcription factors, is conserved in the promoter region of all species. Promoter studies in primary rat

Sertoli cells or a mouse Sertoli cell line displayed lower activities when the E box was mutated (109). Based on the presence of a cAMP-regulatory element (CRE)-like element at around position -115 in the rat FSH-receptor promoter, Monaco *et al.* (110) proposed a role for the inducible cAMP early repressor (ICER) in the regulation of the FSH receptor gene expression. Functional studies in primary rat Sertoli cells showed that the cAMP responsive element modulator (CREM) isoform ICER increases rapidly upon FSH stimulation, indicating that it might be involved in the rapid down-regulation of the FSH receptor transcripts and long-term receptor desensitization. This repression was ascribed to the binding of ICER to the CRE-like sequence in the FSH receptor promoter, since ICER could repress expression of a transcriptional reporter gene containing this CRE-like site in transfected primary rat Sertoli cells. However, the CRE-like motif ATTAGTCA is present neither in the human nor in the mouse FSH receptor promoter, and other rat promoter studies could not demonstrate direct interactions between the CRE-like sequence and rat Sertoli nuclear proteins (109, 111).

The promoter activity was investigated by transfection studies using primary cells and cell lines in which a reporter gene expression vector was driven by different 5'-extensions of the FSH receptor promoter. The highest activity was obtained using constructs of the FSH receptor promoter from -1 to -286 bp relative to the translational start site (105, 106). The activity was markedly reduced when longer DNA constructs were used, indicating the presence of repressor elements. In all cases the FSH receptor promoter was constitutively active in the absence of hormone, and the basal activity of a construct ranging from -847 to +114 was stimulated 4-fold by (Bu)₂cAMP treatment (110). The core promoter region could be allocated to the first 286 bp, a tract that includes the major transcriptional start sites and shows the highest homology among the 5'-flanking regions of the human, rat, and mouse FSH receptor (106). These findings indicate that repression and derepression of *cis*-acting elements upstream of the core promoter region, which mediate constitutive transcriptional activity, is a potential mechanism to modify expression of the FSH receptor promoter activity.

Human FSH receptor promoter activity was observed in cells naturally expressing the receptor, such as human granulosa cells and rat Sertoli cells, but also in the nonexpressing Chinese hamster ovary (CHO) cell line (106). Mouse promoter activity was detected in Sertoli cells but not in CHO cells (107), and rat promoter activity was detected in a mouse Sertoli cell line (MSC-1) and a Leydig cell line (MA-10), but none of the different constructs tested so far was active in COS-7 cells (105). Although the experiments might indicate a cell-specific expression of the FSH receptor gene, which sequences do confer cell specificity have not yet been identified. In transgenic mice carrying a 5-kbp FSH receptor promoter/ β -galactosidase fusion gene, expression of β -galactosidase transcripts was detected only in the testis and the ovary. Thus, elements within this region are able to direct the expression specifically in testicular and ovarian cell types (105). Analysis of cell-specific transcription factors by electrophoretic mobility shift assays revealed several DNA-protein complexes in cells expressing the FSH receptor gene and an additional specific DNA-protein complex in the nonex-

pressing COS-7 cell line (105), indicating inhibition of FSH receptor expression in this cell type.

Promoter methylation might be a mechanism involved in the inhibition of gene expression in cell types other than the cells naturally expressing the FSH receptor. Studies using methylation-sensitive enzymes indicated that DNA methylation of the rat promoter is involved in the suppression of transcription in cells lacking detectable FSH receptor mRNA (105). However, the human and mouse FSH receptor promoters contain neither GC-rich islands nor methylation consensus sequences (CCGG), indicating that methylation events do not play a significant role in the modulation of transcriptional activity in these species.

The absence of usual TATA and CCAAT promoter elements, the presence of multiple transcriptional start sites, and the constitutive expression are features of housekeeping genes (112). Similar characteristics are shared by the promoters of the LH and TSH receptor gene (11, 104), but the overall homology between the core-regulatory sequences of the three receptors is low. The highest homology between the FSH and the LH receptor reaches 72% and is confined to a stretch of 58 nucleotides at position -298 to -352 (100). This is not unexpected, however, since physiological studies indicate that the expression of the two gonadotropin receptors is regulated differently.

Assuming a species-independent general mechanism of regulation of the FSH receptor expression, future studies should carefully elucidate common, non-species-specific sequence motifs in the promoter region of the FSH receptor gene. These elements should then be analyzed *in vitro*, either in primary granulosa and Sertoli cell cultures (keeping in mind the immature status of these cells) or in granulosa or Sertoli cell-derived lines stably expressing a recombinant FSH receptor, to enable FSH stimulation and to mimic, at least partially, the *in vivo* situation. These experiments are crucial to solving the enigmatic regulation of the FSH receptor expression and would have great impact on the targeting of genes specifically to granulosa and Sertoli cells in transgenic studies.

V. Expression of the FSH Receptor and Its Regulation

A. FSH receptor gene expression

The cloning of the FSH receptor cDNA allowed for Northern hybridization analysis of FSH receptor mRNA. It became evident that more than one transcript could be detected in testicular and ovarian tissues. Based on the deduced full-length cDNA sequence, a transcript of approximately 2.5 kb is to be expected. Indeed, such a mRNA transcript is visible in a variety of species (69-74, 79), but several additional bands can be observed as well. Depending on whether total RNA or mRNA preparations are used, at least one larger transcript, in the range of 5-7 kb, and a smaller transcript, in the range of 1.3 to 1.8 kb, can be identified (113). Although the differences in transcript size might be species- and method-dependent, a common pattern of at least three FSH receptor transcripts suggests a non-species-specific mechanism

of gene expression. Furthermore, the transcript pattern is similar in ovarian and testicular tissue.

Studies on the hormonal regulation of the FSH receptor gene expression or during different stages of gametogenesis have shown that the transcripts are not differentially regulated. The ratio between alternative transcripts and the full-length mRNA remains constant (114). The presence of different transcripts has also been shown for the LH and the TSH receptor. However, a short LH receptor transcript (1.3 kb), encoding presumably only the extracellular domain, displays a different pattern of expression regulation compared with the other transcripts (115, 116).

The mechanism underlying the generation of different FSH receptor transcripts might be related either to different transcriptional start sites, or different polyadenylation sites, or to alternative splicing processes. When cDNA libraries were screened with FSH receptor cDNA probes or with RT-PCR, some of the transcripts were isolated and further characterized. However, the use of different transcriptional start sites is not the major mechanism responsible for the generation of transcripts ranging in length from 1.3–7 kb. Rather, the generation of different transcripts seems to originate from different polyadenylation sites giving rise to the long form (5–7 kb) and the normal form (2.5 kb) and, in addition, to alternative splicing of these primary transcripts. Analysis of the nucleotide sequences revealed four different possible mechanisms underlying this isoform heterogeneity:

1. Several isoforms lack one or more exons (79, 117–119). Interestingly, in principle the loss of an exon does not result in changes of the ORF. The isoforms, therefore, encode putative functional receptors. This splicing mechanism is known as cassette-exon-mode and reflects the module-like genomic structure, whereby processes such as insertion or excision of entire exons are enabled by the same exon phasing, leaving the ORF unchanged. This exon shuffling has also been shown for the LH receptor and TSH receptor (120, 121).

2. The second mechanism involves splicing events of the primary transcript through alternative internal 3'-acceptor sites. The presence of conserved splice acceptor sites, *e.g.*, a CAGG nucleotide sequence stretch, can shorten exons if it is located within the exons or lead to incomplete intron splicing (67).

3. Some of the isoforms represent a combination of the cassette-exon-mode and usage of alternative 3'-acceptor sites (68).

4. Another mechanism is represented by the partial retention of intronic sequences. This incomplete splicing generally results in larger transcripts (67, 122, 123).

Splicing events such as described under 2, 3, and 4 in all cases result in a change of the ORF, starting with the branch point. In some cases the new aa sequences show a very basic pattern (122), and the aa sequence is terminated by new termination codons. If the retained introns contain polyadenylation sites, this results in the generation of smaller transcripts. Alternatively, the transcripts are larger than usual.

Apparently alternative splicing processes affect only the extracellular domain of the receptor, encoded by exons 1–9, since no splicing events involving the transmembrane domain encoded by exon 10 have been observed. As most isoforms lack the transmembrane domain whereas the high-

affinity hormone-binding site encoded by the extracellular domain is still present, it is speculated that they might give rise to soluble and secretable receptor fragments. These isoforms could potentially act as hormone-binding proteins and thereby antagonize FSH action by sequestering it in the circulation. Similar hormone binding-proteins deriving from the GH receptor (124), and presumably the TSH receptor (121), have indeed been described and result from receptor shedding or alternative splicing events. However, there is no evidence yet of secreted FSH receptor isoforms. Studies on the LH receptor have shown that isoforms lacking the transmembrane domain are able to bind LH with high affinity but are trapped within the cell (125). A short, truncated TSH receptor form might be expressed and secreted, thereby acting as a nonfunctional autoantigen (121). One possible consequence of the simultaneous expression of the different mRNAs, encoding full and truncated receptor forms, might be a competition for the translation process and thereby regulation of the expression of the mature and functional FSH receptor protein (126).

B. Expression of the FSH receptor in the testis

1. *Localization.* Binding experiments have shown that FSH binds specifically to receptors located on the membrane of Sertoli cells (127, 128). No specific binding was observed in spermatogenic cells, except for an isolated finding suggesting the presence of FSH receptors in spermatogonia (42). Northern blot hybridization experiments in a variety of tissues revealed a distinct signal in the testis, solely in the Sertoli cells (114). *In situ* hybridization confirmed that the Sertoli cells are the only cell type expressing the FSH receptor in the testis (43, 129, 130).

A comprehensive study on FSH receptor expression was performed in a nonhuman primate in which 38 different tissues and organs were screened for the presence of FSH receptor transcripts, using the RNase protection assay technique (131). No transcript could be detected in organs or tissues other than the testis. Thus, unlike the LH receptor, the expression of the FSH receptor seems to be strictly gonad- and cell-specific. Quantification of the FSH receptor mRNA levels in the human and monkey testis showed that 0.05 to 0.1 pg/ μ g testis RNA encode the FSH receptor (132).

Interestingly, using monoclonal FSH receptor antibodies, Vannier *et al.* (44) reported a polar expression of the receptor protein at the basal part of the Sertoli cell and around the spermatogonia. The same group had previously reported that the LH receptor protein can be detected in the vascular endothelial cells of the testis by immunostaining (133). The authors proposed a model in which hCG is transported by receptor-mediated transcytosis from the blood vessel through the endothelium cells to the Leydig cells. This model requires the presence of the LH receptor in endothelial cells. In a recent report the FSH receptor was allocated to small vessels in the interstitial space of the testis, implying a similar transport mechanism for FSH (44). Whether these FSH receptor-like structures represent a fully active FSH receptor capable of signal transduction remains to be shown.

2. *Ontogeny.* Studies of the FSH receptor in the developing testis have demonstrated high-affinity binding for FSH start-

ing from day 28 of gestation in pigs and during the first half and at the end of gestation in primates (134, 135). In the rat, ligand-binding experiments revealed the presence of the FSH receptor from fetal (f) day 17.5 onward. The content of FSH receptor increases between f day 20.5 and birth (136). By Northern blotting and RT-PCR, transcripts encoding the extracellular domain were detected from f day 14.5 onward, and full-length mRNA appeared around f day 16.5. The reason why the extracellular domain can be detected first might be due to differences in the onset of transcription of two mRNA species or, alternatively, the two transcripts may have different half-lives. The FSH receptor is present in the testis before significant concentrations of the cognate hormone appear in the fetal circulation. Furthermore, the fetal testes seem to lack a clear acute cAMP response to FSH despite the presence of the FSH receptor (137). This might be due to an immature signal transduction system or a different FSH receptor coupling in the fetus. From the physiological point of view, this nonresponsiveness might prevent premature activation of FSH-stimulated spermatogenesis (138).

After birth, FSH binding in the mouse testis reaches a peak between days 7 and 21 and then decreases rapidly between days 20 and 37 (139). In the rat, the FSH receptor mRNA increases until day 7, stays constant between days 10 to 20, and drops dramatically around day 40 (140). The initial increase is related both to the increase of receptor numbers per Sertoli cell and to the proliferation of Sertoli cells up to day 10 (141). The subsequent decrease of FSH receptor expression is related to the massive appearance of spermatocytes and spermatids, as indicated by the increasing weight of the testis. The FSH receptor mRNA expression seems to be comparable in adult and immature Sertoli cells (114). The increase of total number and density of FSH-binding sites in the initial phase of testis development has been shown in several mammalian species and in photoperiodic animals (142, 143). Since the receptor increase is parallel to the rise of circulating FSH levels, one might assume that the gonadotropin induces up-regulation of its own receptor in the developmental phase.

3. Regulation of the expression *in vivo*. *In vivo* studies have been performed mainly in hypophysectomized animals, thereby depleted of FSH action, followed by substitution with different hormones. The effects of this treatment on FSH receptor binding is different among rats, mice, and photosensitive animals, such as quails and the Djungarian hamster. In adult quails, FSH binding decreases remarkably in the absence of FSH, suggesting a mechanism of receptor up-regulation by FSH, whereas in mice and rats FSH deprivation leads to an increase in FSH binding, indicating instead a down-regulatory action of the hormone in these species (60). This down-regulation has recently been confirmed in the rat by Northern blot experiments (144, 145). In photoperiodic animals, the day length has a marked effect on the expression pattern of the FSH receptor (142, 143). Animals transferred from short-day to long-day conditions display rapid testicular growth sustained by a pronounced increase in FSH-binding sites induced by the elevation in gonadotropin levels. Since it is assumed that the number of Sertoli cells per testis remains constant, the number of FSH receptors per

Sertoli cell must increase. The only study in humans so far was performed in transsexual men treated with estrogens for sex reversal over long time periods. High-dose estrogen treatment leads to a marked decrease in FSH receptor mRNA levels (132).

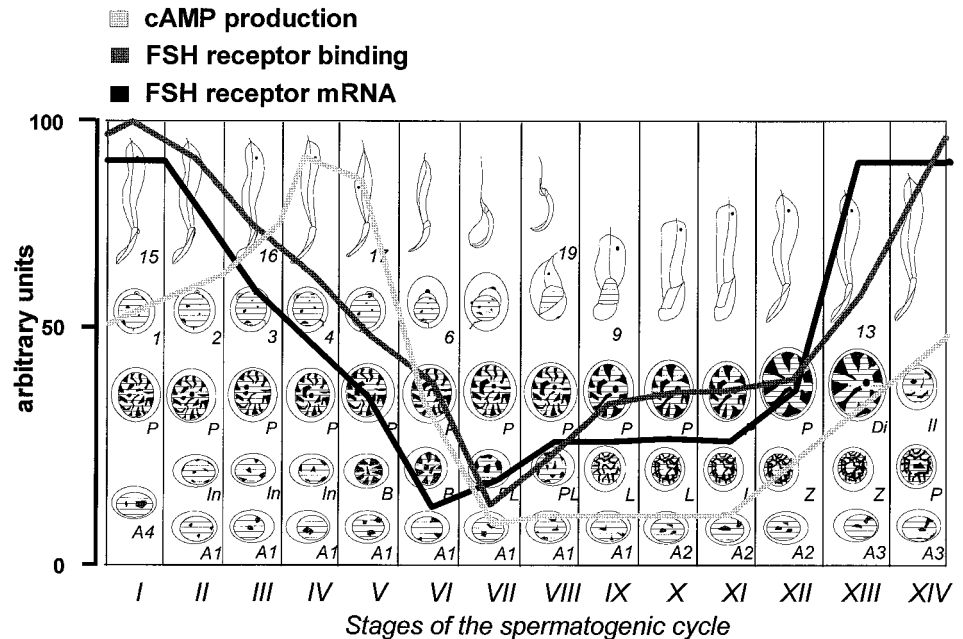
In the rat, spermatogenesis is organized into 14 stages, defined by their different germ cell composition and present simultaneously in different regions along the seminiferous tubules. Dissection of segments containing individual stages or synchronization of spermatogenesis by retinol deprivation and repletion enabled the investigation of FSH receptor expression during the spermatogenetic cycle. FSH binding and FSH receptor mRNA expression studies reach the highest levels in stages XIII, XIV, and I, whereas lowest expression is found in stages VII and VIII (108, 114, 128, 146) (Fig. 5). This stage-dependent expression of the FSH receptor coincides with different maturation states of the germ cells. The more advanced germ cells, *e.g.*, those in stage VI and VII when spermiation occurs, colocalize with reduced FSH receptor expression, whereas stages containing less advanced germ cells, such as early spermatids, are colocalized with an increased expression. FSH-stimulated cAMP production in isolated sections of seminiferous tubules is highest in stages II to IV (Fig. 5), indicating further regulation of signal transduction by local factors (128, 147). Overall, these data indicate that a stage-specific paracrine interaction between spermatogenic cells and Sertoli cells regulates FSH receptor expression (148).

4. Regulation of expression *in vitro*. Most *in vitro* studies were performed using immature rat Sertoli cells. A disadvantage of such studies is the lack of interaction between Sertoli cells and the different germ cells. Cocultures of germ and Sertoli cells are extremely difficult to perform. Treatment of immature Sertoli cells with different doses of FSH results in a marked decrease in FSH binding and mRNA expression within 4–8 h. The mRNA levels recover to normal after 24 h, whereas FSH binding decreases further (144, 145). The effect of FSH treatment on the mRNA levels can be mimicked by (Bu)₂cAMP. The diverging effects on binding and mRNA levels suggest that the early effect of FSH on its receptor involves sequestration, *i.e.*, receptor internalization, followed by a slower down-regulation mediated at the posttranscriptional level by cAMP. No data are available so far concerning the effects of FSH on receptor mRNA stability, a possible mechanism for the effects observed (108).

C. Expression of the FSH receptor in the ovary

1. Localization. In the female, FSH binding has been localized to the granulosa cells (149, 150). Several recent studies using molecular biology techniques confirmed that the granulosa cells are the only cell type expressing the FSH receptor (119, 151–153). Thus, as in the male, the expression of the FSH receptor in the female is strictly gonad- and highly cell-specific. This finding is in contrast to the expression pattern observed for the LH receptor and TSH receptor. LH receptor expression can be demonstrated in a variety of organs and tissues (154), and the TSH receptor expression has also been shown in extra-/retro-orbital tissue (121), suggesting hitherto

FIG. 5. Stage-specific expression of the FSH receptor in the rat during spermatogenesis. In the rat, spermatogenesis is organized into 14 stages, constituting the spermatogenic cycle of the seminiferous tubule. The different stages are defined by their different germ cell compositions. All stages are present simultaneously in different regions along the tubules. A1–A4, Spermatogonia; In, B, Pl, L, Z, P, Di, different stages of the first meiotic division of the spermatocytes; II, second meiotic division of the spermatocytes; 1–19, maturation and elongation of the spermatids. Expression levels of the FSH receptor are given in an arbitrary scale ranging from 0–100 U.



unknown or only suspected physiological functions of LH and TSH in other tissues. Similarly, a recent report indicated the presence of FSH receptor protein and mRNA in cultures of human myometrial smooth muscle cells (155). However, this isolated finding awaits further confirmation.

2. *Ontogeny.* The acquisition of FSH receptors is essential for granulosa cell differentiation and for follicle maturation (156). In the fetal rat ovary, expression of the extracellular domain of the FSH receptor is first detected on day 20.5. Full-length transcripts appear later, on day 1 post partum and more clearly from day 5 onward in the rat and mouse (130, 157). Similar to the ontogeny of the FSH receptor expression in the testis, this sequential appearance of short and full-length transcripts might reflect differences in mRNA half-lives and/or differences in the onset of transcription of the two RNA species. High-affinity binding sites are present on granulosa cells from day 3 onward, and a constant increase is observed until day 21 when the expression reaches a plateau (158, 159). In general, there is a strong parallelism in the developmental changes in FSH receptor mRNA and receptor protein (158, 159). The ovary does not respond to FSH between birth and day 3, whereas from day 4 to 7 ovaries show an acute, FSH-sensitive cAMP response coincident with the appearance of full-length FSH receptor mRNA (130, 149, 158).

3. *Regulation of expression in vivo.* In the immature rat ovary FSH receptor mRNA can already be localized in the granulosa cells of small follicles. Treatment with PMSG to stimulate follicle growth results in a marked increase of FSH receptor mRNA expression and FSH-binding sites, whereas subsequent administration of hCG to induce ovulation and luteinization significantly decreases FSH receptor expression (160, 161). In hypophysectomized, estrogen-treated rats, follicular development and ovulation can be induced by FSH alone (162), which increases both FSH binding and FSH receptor mRNA levels. In contrast, induction of ovulation

with a surge dose of recombinant FSH suppresses FSH binding and FSH receptor gene expression. These data suggest a biphasic, homologous regulation of FSH receptor expression in the ovary. Low doses of FSH increase the number of FSH-binding sites parallel to the increase of FSH receptor mRNA levels. High doses of FSH down-regulate FSH receptor-binding sites and mRNA levels, suggesting a suppression of gene expression and protein synthesis concomitant to the increased receptor occupancy and internalization (116, 160). This biphasic mechanism might be due either to changing mRNA stability in the presence of different hormone concentrations or to effects on the regulatory elements in the promoter region of the FSH receptor gene. However, analysis of the FSH receptor promoter does not give any further clues, since no obvious regulatory elements can be detected therein (see Section IV). Whether the recently identified transcription factor ICER (110) can interact with promoter regions of the FSH receptor, and thereby be involved in the biphasic regulation, remains to be shown.

Examination of sexually mature adult rats during the 4-day estrous cycle revealed the presence of FSH receptor in nearly all follicles starting with only one layer of granulosa cells (151). The levels seem to rise during follicular maturation, although some reports claim that the steady state FSH receptor levels in healthy follicles do not correlate with follicular size (163–166), and decrease drastically in the post-ovulatory follicle, after the LH surge (Fig. 6). With luteinization, FSH binding can no longer be detected. In the bovine, full-length FSH receptor transcripts are still detectable 1 day after luteinization, albeit at low levels. By day 3, full-length transcripts are no longer detectable but, surprisingly, expression of the extracellular domain persists (117). This specific pattern of loss of FSH receptor gene expression resembles, in reverse, the onset of expression in the ovary, again suggesting that the two transcripts might have a different half-life and/or be produced in a regulated succession. Follicular atresia is associated with decreased responsiveness to

FSH and reduced mRNA receptor levels, due to a transcriptional down-regulation or decreased stability of receptor mRNA (119, 165, 167). Although FSH receptor expression has long been recognized to be under the control of FSH, a recent study in hypogonadal mice lacking circulating gonadotropins revealed the presence of FSH receptor mRNA in the ovary. Thus, factors other than FSH may act on the induction of FSH receptor expression (156).

FSH receptor expression in granulosa cells of developing follicles remains constant in the first half of pregnancy and increases during the second half (150, 168). Such an increase may be necessary for the development of follicles, although it appears to be independent of FSH but dependent on other unknown factors (168, 169). In humans, studies in perimenopausal patients with irregular cycles revealed a correlation between increased FSH serum levels and decreased FSH receptor (170). This change seems to be parallel to the changes observed in follicle morphology and number. In postmenopausal patients, FSH receptors are no longer detectable (170).

4. Regulation of expression *in vitro*. Granulosa cells of immature, estrogen-treated rats contain FSH receptors that decline during culture. Treatment with FSH maintains the expression, suggesting that FSH increases the levels of its own receptor. Estrogens synergize with FSH *in vitro* to increase the number of receptors per granulosa cells, but alone do not alter the expression (152). The FSH-related increase of FSH receptor expression is dose-dependent and can be mimicked by the adenylyl cyclase activator forskolin and by cholera toxin, indicating that the gonadotropin can amplify its own action on granulosa cell differentiation and maturation. The suppression of FSH receptor reported *in vivo* by an ovulatory dose of FSH (116), however, is not observed in cultured granulosa cells. This discrepancy might be due to the absence *in vitro* of paracrine factors involved in the regulation of the FSH receptor expression *in vivo* or might be merely related to the immature status of the granulosa cells.

Paracrine factors are involved in the regulation of FSH receptor expression. Treatment of granulosa cells with epidermal growth factor, basic fibroblast growth factor, or insulin-like growth factor-1 attenuates the response to FSH but does not alter basal levels of expression, whereas GnRH completely suppresses the induction of FSH receptor mRNA by FSH (152). Other growth factors, such as transforming growth factor- β and activin, are potent inducers of FSH receptor expression (159, 171–173). In the presence of FSH,

activin has a biphasic action, which is inhibitory at low doses and stimulatory at high doses (174). The mechanism whereby transforming growth factor- β and activin increase FSH receptor expression is still not clear. By acting via tyrosine kinase receptors, they do not directly increase intracellular cAMP accumulation, and it is therefore reasonable to assume two distinct pathways of FSH receptor induction.

In porcine granulosa cells not previously exposed to estrogens, the FSH receptor increases with time in the absence of FSH. FSH causes a dose-dependent decrease in FSH receptor binding, while stimulating FSH receptor mRNA levels (113). This differential regulation of protein and mRNA levels would not be mediated by cAMP, since cholera toxin increases FSH receptor both at the protein and mRNA level (166). However, the decrease of FSH binding might be due to the blocking of binding by unlabeled FSH and/or the FSH receptor is internalized and degraded as a consequence of FSH binding.

Future studies should clarify the biphasic mRNA expression in the estrous cycle. The identification of regulatory elements in the promoter region of the FSH receptor gene is therefore necessary. Paracrine factors and/or intracellular repressors involved in stage-specific receptor expression during the spermatogenic cycle should be identified in the male. In particular, it will be interesting to analyze the receptor expression in male infertility, especially considering that mutations of the FSH receptor are obviously rare in this condition (see Section X). To this end, sensitive detection methods capable of quantifying expression in biopsy material must be developed.

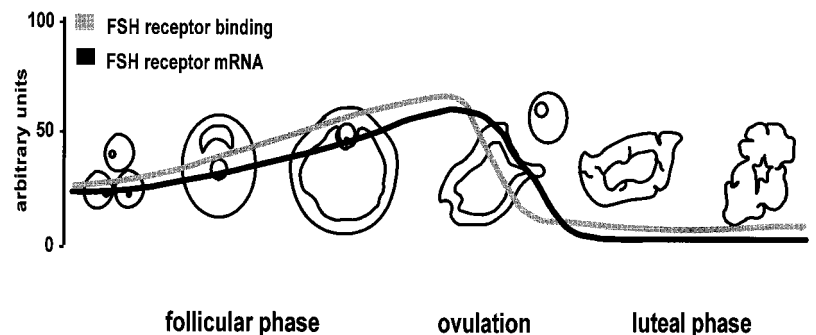
VI. Expression of the FSH Receptor in Cell Lines

After cloning of the cDNA, the FSH receptor was expressed in a number of cell lines. Mostly, the rat and the human receptor cDNA were successfully introduced into eukaryotic cells and shown to be functional, producing a number of recombinant lines useful for establishing novel FSH bioassays and for studying FSH receptor properties such as hormone binding, signal transduction, and desensitization (Table 2, including Refs. 175–183). Among the cloned FSH receptors from other species, the ovine and the porcine receptor were expressed as well (72, 73).

A. Cell lines expressing the recombinant FSH receptor

Table 2 summarizes the data obtained from the expression of the rat and human FSH receptor in different cell types.

FIG. 6. Expression of the FSH receptor during estrus. Follicle maturation is indicated by the development of primary follicles into a Graafian follicle. After ovulation the corpus luteum and the corpus albicans are shown. Expression levels of the FSH receptor are given in an arbitrary scale ranging from 0–100 U.



With the exception of one line obtained from immortalized granulosa cells (181), the cells used do not derive from progenitors normally expressing the FSH receptor, but possess functional G_s to which the recombinant receptor couples.

In vitro expression is obtained by transfection of an expanding cell population with a suitable vector where the receptor cDNA is placed under the control of a strong promoter. As shown in Table 2, the degree of expression, in terms of number of receptors per cell, varies consistently between the different lines, and this variability seems to be relatively independent of the type of promoter driving the transcription. A factor potentially limiting the expression of the FSH receptor cDNA is the above mentioned presence of several stop codons in the 5'-untranslated sequence immediately preceding the translational start site. The modification of this tract of sequence (183) or the insertion therein of artificial intronic sequences (67) has been a useful strategy for improving receptor expression.

In all the cell lines produced, the FSH receptor is coupled to G_s and adenylyl cyclase, and the exposure of the recombinant cells to FSH leads to a saturable, dose-dependent cAMP production. As shown in Table 2, the ED_{50} of FSH-dependent cAMP accumulation varies impressively between the various cell lines and is little related to differences in receptor density. Since the direct comparison of the ED_{50} values is hampered by the use of different experimental systems, *i.e.*, cells, constructs, and FSH preparations (184, 185), the reasons for this variability are not clear. Future experiments should explore the possibility that part of the observed differences might be due to a different coupling efficiency to adenylyl cyclase in the different cell lines. The K_D values obtained from binding studies with recombinant receptors are usually in the nanomolar range, yet 10- to 1000-fold higher than those found with membrane preparations of native rat and calf receptors (56). However, FSH receptor binding is known to be influenced by many factors, including temperature, ionic strength (186, 187), and time (188), and the direct comparison of the properties of native and recombinant receptors under the same experimental conditions has not yet been performed.

The first expression studies of the human FSH receptor cDNA suggested that there might be a certain degree of species specificity in the hormone-receptor interaction. In fact, the human recombinant receptor transiently transfected in 293 cells was shown to interact more effectively with human FSH compared with rat FSH and, even more, to FSH from other species. In contrast, both human and rat FSH showed similar affinity to rat receptors obtained from a crude preparation of testicular membranes, with the FSH preparations from other species having lower affinity (78). However, the species specificity of the two receptors was shown to be similar when both rat and human FSH receptor were of recombinant origin (176). Thus, the issue of species specificity is not clear, while the experimental approach used in these studies may not be completely appropriate. In fact, the FSH-receptor interaction was compared using either recombinant human receptors *vs.* native rat receptors (78) or recombinant receptors expressed in different cell types (176). Moreover, the presumably uneven purity of the FSH preparations from different species might have influenced the

estimation of the ED_{50} values. The precise determination of species specificity requires an experimental setting based on recombinant human and rat receptors expressed at the same level in the same cell type, where displacement is obtained by known concentrations, expressed in molar terms, of highly purified, ideally recombinant gonadotropin. However, both recombinant and native rat FSH receptor seem to have the same affinity for rat and human FSH, confirming the validity of the results obtained by traditional *in vitro* bioassays based on rat granulosa and Sertoli cells (189).

B. Measurement of FSH by means of "recombinant" in vitro bioassays

Recombinant cell lines permanently expressing the FSH receptor have been used in the development of *in vitro* bioassays (Table 3) but the overall low sensitivity remains a major limiting factor for assessing low serum FSH concentrations. One way to improve sensitivity has been the co-transfection of a reporter gene controlled by a CRE (68, 78). In response to FSH, the increase of intracellular cAMP stimulates the expression of the reporter gene, whereby signal amplification is expected. To this purpose, luciferase was placed under the control of cAMP-responsive sequences of the rat tissue plasminogen activator promoter (78) or of the gonadotropin α -subunit promoter (68). The signal amplification, however, has been disappointingly low, yielding only a 2-fold improvement in ED_{50} in 293 cells (78) and an 3.8-fold improvement in CHO cells (68), underlying the crucial role of the cells' own transcription factors. Another approach has been the permanent expression of the FSH receptor in steroidogenic cells. It is well known that granulosa and Sertoli cells rapidly lose receptor expression when maintained *in vitro* for a long time or when immortalized (181, 190). After reintroduction of the recombinant rat FSH receptor in immortalized granulosa cells (181) or of the human FSH receptor in the adrenal cell line Y1 (67), the recombinant cells responded to FSH with a dose-dependent production of progesterone with a decrease in the ED_{50} up to 70-fold compared with cAMP measurement (67). None of these lines, however, has yet been used for the assessment of FSH in serum.

Until now, only two cell lines sensitive enough for serum FSH bioassay have been thoroughly characterized and validated: an L cell line bearing the recombinant rat FSH receptor (183) and a CHO cell line with the human FSH receptor and luciferase as reporter gene (191). Notably, without reporter gene, the L cell line FSH receptor 7/12 displays the same sensitivity as the CHO cell line, suggesting a very efficient coupling of receptor and effector system. These two bioassays have been used for the measurement of bioactive serum FSH in normal and hypogonadal men (183), in fertile and postmenopausal women (183, 192), and in infertile patients with Sertoli-cell-only syndrome (193). A third bioassay, less well characterized, based on CHO cells with the recombinant human FSH receptor, has been applied to serum FSH in postmenopausal women and in patients with premature ovarian failure (POF) (191). Finally, the Y1 line (67) has been employed to search for Igs blocking the FSH receptor in women with POF (194).

This limited experience is insufficient to establish whether

TABLE 2. Expression of the FSH receptor in cell lines

| Cell line (origin of the cells) | FSH receptor | Promoter | Receptor number (per cell) | Functional characteristics | | | K _d (nM) | Reference |
|--|--|-------------|----------------------------------|----------------------------|------------------|-----------------------|------------------------|------------------|
| | | | | Outcome measure | ED ₅₀ | FSH used | | |
| 293 (Human embryonic kidney) | Rat, cDNA | CMV | 260,000 | cAMP | 80 pM | NIDDK, oFSH-17 | 1.7 | 6 |
| | Rat, cDNA | CMV | | cAMP | 10 ng/ml | NIDDK, oFSH-17 | | 175 ^a |
| | Rat, cDNA | CMV | | FSH binding | | | | 78 |
| | Rat, cDNA | CMV | | FSH binding | 2.1 ng/ml | NIDDK-hFSH-1-3 | 3.1 | 176 ^a |
| | Rat, cDNA | CMV | | FSH binding | 3.1 ng/ml | NIH-rFSH-RP-2 | | 176 ^a |
| | Rat, cDNA | CMV | | FSH binding | | NHPP-hFSH-AFP-5720D | 0.87 | 85 |
| | Rat, cDNA | CMV | | cAMP | 2.9 ng/ml | NHPP-hFSH-AFP-5720D | | 177 ^a |
| | Rat, cDNA | CMV | | IP | 109 ng/ml | NHPP-hFSH-AFP-5720D | | 177 ^a |
| | Rat, cDNA | CMV | 406,000 | cAMP | 2.5 ng/ml | NHPP-hFSH-AFP-5720D | | 178 ^b |
| | Rat, cDNA | CMV | 406,000 | FSH binding | | NHPP-hFSH-AFP-5720D | 9.3 | 178 ^b |
| | Human, cDNA | CMV | 11,800 | cAMP | 780 pM | NHPP-hFSH-AFP-5720D | | 179 |
| | Human, cDNA | CMV | 11,800 | FSH binding | | NHPP-hFSH-AFP-5720D | 2.81 | 179 |
| | CHO (Chinese hamster ovary) | Human, cDNA | MMT-1 | 16,000 | cAMP | 140 IU/liter | hFSH, recombinant | 2.76 |
| Human, cDNA | | SV40 | cAMP | | 190 IU/liter | WHO, 71/223 | 68 | |
| Human, cDNA | | SV40 | FSH binding | | | WHO, 71/223 | 68 | |
| Human, cDNA | | SV40 | | cAMP | 50 ng/ml | NIDDK-hFSH-I-3 | | 77 |
| Human, cDNA | | SV40 | | FSH binding | | NIDDK-hFSH-I-3 | 0.62 | 77 |
| Human, cDNA | | SV40 | | FSH binding | 11.8 ng/ml | NIDDK-hFSH-I-3 | 1.6 | 176 |
| Human, cDNA | | MMT-I | | FSH binding | 181 ng/ml | NIH-rFSH-RP-2 | | 176 |
| Human, cDNA | | | 3,000 | FSH binding | | hFSH, recombinant | 0.025 | 180 |
| Human, cDNA | | | 3,000 | cAMP | 24.9 IU/liter | hFSH, recombinant | | 180 |
| COS-7 (Monkey kidney) | Human, cDNA with in- tronic sequences | MMT-I | | | Not determined | hFSH, recombinant | | 67 |
| Y1 (Mouse adrenal) | Human, cDNA with in- tronic sequences | MMT-I | | cAMP | 280 IU/liter | hFSH, recombinant | | 67 |
| | Human cDNA with in- tronic sequences | MMT-I | | Progesterone | 4 IU/liter | hFSH, recombinant | | 67 |
| Immortalized granulosa cells (immature rat) | Rat, cDNA | SV40 | 27,000 | FSH binding | 45 pM | NIDDK, oFSH-14 | 0.107 nM | 181 |
| | Rat, cDNA | SV40 | 27,000 | Progesterone | 200 pM | NIDDK, oFSH-14 | | 181 |
| Sf9 Insect (baculovirus system) | Human, cDNA | Polyhedrin | 7,000 | FSH binding | 2 ng/ml | hFSH, recombinant | 0.17 | 89 |
| Sf9/Hi5 insect (baculovi- rus system) | Rat, cDNA | Polyhedrin | 152,000–317,000 | cAMP | 1.24 nM | hFSH, own preparation | | 182 |
| | Rat, cDNA | Polyhedrin | 152,000–317,000 | FSH binding | | hFSH, own preparation | 0.013–0.031 | 182 |
| ltk ⁻ mouse fibroblast | Rat, cDNA with 5' modi- fication | SV40 | 10,000 | cAMP | 10 IU/liter | WHO 78/549 | | 183 |
| | | SV40 | 10,000 | FSH binding | | WHO 78/549 | 1.42 | 183 |

^a Same cell line [293(wt-1)] used in different laboratories.^b Line 293(wt-10).

TABLE 3. Available bioassays based on recombinant FSH receptor cell lines used for determination of serum FSH

| Cell line | Type of FSH receptor | Reporter | Outcome measure | Standard FSH | ED ₅₀ | Sensitivity (IU/liter) | | | Reference |
|-------------------------|----------------------|------------|-----------------|----------------|------------------|------------------------|------------|---------------------------------|-----------|
| | | | | | | Without serum | With serum | Maximal serum concentration (%) | |
| FSH 7/12 | Rat | None | cAMP | WHO 78/549 | 10 IU/liter | 0.3 | 3 | 10 | 183 |
| CHO-FSHR (from Ref. 75) | Human | None | cAMP | NIDDK-hFSH-I-3 | Not determined | 0.6 | 6 | 10 | 191 |
| CHO-FSHR (from Ref. 67) | Human | Luciferase | Luminescence | WHO 71/223 | 7.7 IU/liter | 1 | 4 | 25 | 68, 192 |

the use of the homologous, human FSH receptor discloses features of FSH bioactivity overlooked by the use of the rat receptor or the traditional bioassays. Interestingly, in normal women, the homologous bioassay revealed a previously unrecognized increase in the relative gonadotropin bioactivity in the late luteal phase (192). An interesting, ongoing development is the reintroduction of the FSH receptor in Sertoli cell lines (195).

C. FSH receptor function in cell lines

Apart from the development of bioassays, the rat and human FSH receptor cDNA have been transiently or permanently expressed in several cell types, as indicated in Table 2, with the aim of investigating some aspects of receptor physiology. In the following sections of this article we will discuss in detail the studies based on recombinant receptor, addressing the issues of hormone binding, receptor activation, and signal transduction (85, 86, 175, 179, 182, 196), as well as receptor desensitization (77, 177, 178, 197). The efficient expression in the baculovirus system represents an alternative approach to the creation of permanent cell lines when the effects of several constructs or mutations have to be investigated (182). Receptor peptides have also been produced in *Escherichia coli* for binding studies (44, 198).

The increased experience of several investigators studying the FSH or other G protein-coupled receptors in cell lines is now leading to a better appreciation of potentials and limitations of these systems. For example, the transfected receptor could be coupled, with varying efficiency, to the effector system in different cell types. This might affect the study of receptor activation. For instance, unlike the cognate LH and TSH receptors, the FSH receptor bearing the Asp 567 Gly mutation shows little constitutive activity in COS-7 cells (8) and in 293 cells (199), whereas constitutive activity is better appreciated in another cell line derived from Sertoli cells (see Section X). Moreover, in cell lines the receptor might become coupled to unusual effector pathways (177, 200). Future experiments of structure-function relationship with recombinant FSH receptors should consider these aspects and, in particular, analyze whether and which cell system produces results really meaningful to clinical practice.

VII. Structure-Function Relationships and Models of FSH-FSH Receptor Interaction

A. General features

The FSH receptor is synthesized in granulosa and Sertoli cells and transported to the membrane surface. Mutations that prevent receptor folding and/or transportation result in

the retention of the receptor protein within the cell (196). During synthesis, the receptor is glycosylated, and this process is necessary for the proper folding of the nascent protein and for its expression at the plasmalemma (85, 196). There are four putative glycosylation sites in the extracellular domain of the human FSH receptor and three in the rat FSH receptor. Data obtained with the recombinant rat receptor, expressed in 293 cells, suggest that two of the three potential sites might be actually N-glycosylated, *i.e.*, Asn 191 and Asn 293 (85). The sugar moieties, however, are not necessary for hormone binding (85, 201). Both the rat FSH receptor naturally expressed in isolated Sertoli cells and the recombinant human receptor expressed in CHO cells are found distributed over the entire cell surface, in clumps or patches (182, 202).

Studies with the recombinant FSH receptor invariably indicate that it is a monomer of a molecular size expected from the primary structure (85, 90, 182, 196). In contrast, ligand blotting and Western blot analysis of the FSH-binding sites obtained from calf and rat testicular membranes assign much larger dimensions to the immunoreactive material, with a molecular mass of 240 kDa, reduced to 60 kDa under denaturing conditions (28, 57, 201–203). To reconcile these findings, it has been postulated that the receptor protein might undergo posttranslational modifications and that the mature FSH receptor is an oligomer stabilized by a disulfide bond (57).

Although earlier studies suggested that the gonadotropin receptors might have a dimeric (204) or trimeric (54) structure, such a quaternary organization has not been confirmed using recombinant techniques (9). In this respect, the LH and FSH receptors seem to be different from the TSH receptor, which consists instead of two subunits (205) and can release the extracellular domain as soluble protein (206). While a few reports indicate that the binding domain of the gonadotropin receptors might be secreted in the extracellular space (207, 208), the most recent results obtained with truncated forms produced in mammalian cells do not support this view (85, 179, 196, 209).

B. Structure-function relationships

1. *Binding determinants.* While there is no doubt that interaction with the hormone requires the large extracellular part of the receptor, the questions to be answered are which residues are important and whether the transmembrane domain also participates in hormone binding. Three different approaches have been used to address the problem of the binding determinants of the FSH receptor: construction of chimeric receptors, site-directed mutagenesis, and synthetic peptides.

The classic study with chimeric LH/FSH receptors of Braun *et al.* (86) suggested that the specificity for FSH is conferred by a tract of the extracellular domain including the LRR 1–10, *i.e.*, N-terminal to aa position His 274. More precisely, the specificity might be localized between Phe 165 in repeat 5 and His 274 in repeat 10 (see Figs. 2 and 3), because a LH receptor chimera including FSH receptor repeats 1–10 responded to FSH but not to hCG, while a FSH receptor chimera containing LH receptor repeats 1–6 responded only to hCG. (86). This study also suggested that the transmembrane domain does not confer hormone specificity. Another study showed that a truncated form consisting only of the extracellular domain of the FSH receptor was not secreted, but could be recovered in cell lysates and bound [¹²⁵I]FSH with a dissociation constant (K_d) comparable to that of the wild type receptor (179). It was also shown that His 424 in the first extracellular loop of the transmembrane domain is important for high-affinity binding, because when mutagenized to Ala, the affinity of the receptor decreased 4-fold (179). The oligosaccharides in the extracellular domain do not seem to influence FSH binding, and their enzymatic cleavage has no effect on high-affinity hormone binding (85).

Collectively, these results show that hormone specificity is confined to the extracellular domain of the FSH receptor, which contains the binding site(s). The transmembrane domain might contribute to high-affinity binding, but not necessarily. Attempts to identify in more detail which tracts of the extracellular domain are involved in high-affinity, specific binding are based largely on a synthetic peptide strategy. Comparison with other glycoprotein hormone receptors allows identification of portions in the extracellular part with little homology that possibly participate in hormone binding. Synthetic peptides corresponding to these tracts are then tested for their ability to bind FSH and compete with the whole FSH receptor for hormone binding. This approach, however, does not consider the spatial configuration of the peptides, which might be substantially different from that of the entire receptor and thereby produce nonspecific effects. For example, when larger recombinant peptides are used, the binding properties of shorter tracts contained therein cannot be reproduced (198). Another important limitation of the studies based on synthetic peptides is that they are often active only in milligram concentrations, and the competitive nature of their effect on binding inhibition is not convincingly demonstrated, thereby leaving the issue of specificity unresolved. Alternatively, synthetic peptides are used to raise antisera that, in turn, are assayed for their capacity to recognize the whole receptor. Based on these criteria, two putative binding sites were identified that do not correspond to those indicated by the chimeric receptor strategy (86): a short tract within the first 47 N-terminal aa residues (90, 203, 210) and a longer, less accurately definable region situated closer to the C terminus between aa position 218 and 332 (182, 198, 211).

In summary, virtually two-thirds of the extracellular receptor domain has been proposed, by various approaches, to be involved in hormone receptor interaction. Clearly, only the crystal structure of the hormone-receptor complex will define the actual binding site, but, due to the large dimensions of such a complex, this information is difficult to obtain.

Models of FSH-receptor interactions might be useful in the meantime (212–215).

2. *Signal transduction.* In contrast to the related TSH receptor and LH receptor (10), information about the FSH receptor determinants involved in signal transduction is scant. The experimental strategies used to address this issue include both synthetic peptides and site-directed mutagenesis, whereas only few naturally occurring mutations have been reported to date.

Grasso *et al.* (216, 217) constructed synthetic peptides corresponding to the aa positions 533–555 and 645–653 of the rat receptor and tested their activity on cAMP and estradiol production by immature rat Sertoli cells *in vitro*. The peptides were selected on the basis of their homology to structural determinants of signal transduction in other G protein-coupled receptors, which have been shown to include at least two basic residues at the N terminus and possess a BBXXB or BBXB motif (where B is a basic and X a nonbasic aa). The peptides did not inhibit FSH binding and could stimulate cAMP and estradiol secretion. Although the participation of these regions of the FSH receptor to signal transduction is highly probable, based on structural resemblance to relevant segments of the G protein-coupled receptors, the adequacy of the experiments employed is questionable. For example, the region in the human FSH receptor corresponding to the peptide 645–653 does not include the BBXB motif. Moreover, the stimulation of cAMP and estradiol production was not impressive, and the specificity of the peptides was not shown (216, 217). Also the approach of peptide analogs containing additions intended to change the overall charge of this receptor domain does not clarify the issue (218).

Experiments based on site-directed mutagenesis have shown that the substitution of residues Asp 422, Thr 425, and Lys 426 of the first extracellular loop of the transmembrane domain of the human FSH receptor, in immediate continuity with the second transmembrane segment, leads to loss of receptor function in the presence of increased binding affinity. Instead, substitution of His 424 reduced binding affinity and increased receptor activation, showing that hormone binding and signal transduction are separate properties of the receptor (179). These results, however, are completely at odds with those published simultaneously by Rozell *et al.* (196). These authors found that the same Ala substitution of the amino acid Asp 404 of the rat FSH receptor, corresponding to Asp 422 in the human FSH receptor, resulted in complete loss of binding activity and intracellular trapping of the receptor in the endoplasmic reticulum (196). Both research groups used the same expression system, *i.e.*, human kidney 293 cells, and the reason for this important discrepancy is not immediately clear. One difference between the human and the rat FSH receptor is that the latter lacks a Glu residue at position 316. Moreover, in the immediate vicinity, the human FSH receptor possesses a putative glycosylation site (Asn 318) not present in the rat receptor. In the experiments of Rozell *et al.* (196) the binding properties of the rat FSH receptor were also lost when Asp 404 was substituted by aa other than Ala and when the receptor was truncated at position 637 in the cytoplasmic tail, a position that should not directly affect hormone binding. Since similar substitutions

in the rat LH receptor had no effects on binding, the authors concluded that mutated FSH receptors retained in the endoplasmic reticulum do not undergo proper folding and are incapable of hormone binding even when a hormone-binding site has not been altered. Whether the sequence differences between the rat and human receptors can justify these discordant findings remains to be determined, but, if so, the area around aa position 316 might be located three-dimensionally in close proximity to the first extracellular loop.

Finally, the importance of the third intracytoplasmic loop for signal transduction is proven by the recent description of an activating mutation of the FSH receptor leading to an Asp → Gly transition at position 567 (see below).

C. Models of FSH-FSH receptor interaction

1. *Binding determinants on FSH.* Working out a model of gonadotropin-receptor interaction must take into account not only the putative binding determinants on the receptor but also the contact points on the hormone. The crystal structure of hCG has revealed that glycoprotein hormones are dimers of Y-shaped subunits stabilized by three central disulfide bonds forming a cysteine knot and held together by a segment of the β -subunit that wraps around the α -subunit like a seat belt (219, 220). Several approaches have been employed to identify binding determinants on FSH, including epitope mapping, use of synthetic peptides and antipeptide antibodies in *in vitro* bioassay and receptor assays, enzymatic modifications of gonadotropins, and site-directed mutagenesis. The putative binding regions on FSH identified by using these methods (Refs. 211, 221–239; partially reviewed in Refs. 240–242) include at least two regions in FSH β that might be involved in receptor binding, roughly FSH β 30–60 and FSH β 80–110. In the three-dimensional structure of hCG, these regions would correspond to the long loop (second loop of the β -chain, or L β 2) and determinant loop/seat belt region. However, many data demonstrate that parts of *both* subunits participate in the binding (Refs. 230 and 237 and references therein), an aspect neglected by the synthetic peptide approach. A current model based on data obtained by a combination of synthetic peptide and mutagenetic approaches identifies the binding facet of FSH in a discontinuous functional epitope including the second and the third loop of the α -subunit (L α 2, L α 3) and the third loop of the β -subunit (L β 3) (242). Moreover, receptor binding and signal transduction are clearly distinct properties of FSH (234), the latter being dependent on the integrity of the glycosylation site α 52 (234, 243). The studies with chimeric gonadotropins have also shown that, although the specificity of each gonadotropin for its receptor may be conferred by particular aa, contact points and binding site of hormone and receptor, respectively, must be very similar among the members of the glycoprotein hormone/receptor family (237). In the case of FSH, aa residues on the binding surface belonging to the α -subunit would be important for binding and stabilization of FSH-receptor interaction, while the residues on the β -subunit confer specificity as well as provide binding energy (242).

An alternative strategy to identify residues involved in receptor binding is based on a process of elimination seeking

mutations that do *not* alter hormone binding and signal transduction and binding sites of monoclonal antibodies recognizing *receptor-bound* hormone. By doing this, Cosowsky *et al.* (244) showed that the residues in the third and first loops of the β -subunit (L β 1, L β 3) adjacent to the second loop of the α -subunit (L α 2) make contact with the receptor and that the conformation of the hormone changes during binding. By epitope mapping of recombinant hCG-LH receptor complexes with monoclonal antibodies, Remy *et al.* (215) identified the binding site in a region including the interface of α - and β -subunit, between the C-terminal part of the seat belt and L α 2, whereas L β 1 and L β 2 would be freely accessible and thereby not involved in the hormone-receptor contact. Collectively, these data indicate that while the binding surface of gonadotropins has been approximately identified, its exact extension remains to be determined.

2. *Models of interaction.* According to the first model proposed by Braun *et al.* (86), receptor activation by gonadotropins might occur in two possible ways. The first possibility assumes that the hormone makes contact only with the large extracellular domain of the receptor, which undergoes a conformational change resulting in activation of the membrane-spanning segments. In this model the hormone binding to the receptor-binding site suffices to induce receptor activation. Alternatively, in the “tether” model, hormone binding and receptor activation are determined by different sequences and the hormone bound to the receptor interacts with another activation site, probably in the transmembrane segment. The tether model was favored because it considered a function for both hormone subunits, was closer to the activation model of other G protein-coupled receptors for small ligands, and could better reconcile the structure-function data thus far obtained (reviewed in Ref. 245).

In the ensuing years, the probable arrangement of the seven-transmembrane helices was deduced by analogy with the structure of bacteriorhodopsin (246) and rhodopsin (247), and from extensive computer-aided comparison and alignment of hundreds of G protein-coupled receptors (88, 248, 249). Another major development was the resolution of the crystal structure of the ribonuclease inhibitor, a protein with LRR that can be considered a prototype for the extracellular domain of the glycoprotein hormone receptors (83).

The model of Moyle *et al.* (213) was obtained by modifying the structure of the RNase inhibitor according to the sequence of the LH receptor and subsequent docking of this new, three-dimensionally arranged extracellular domain onto the structure of bacteriorhodopsin. The hormone was then accommodated in the receptor by presenting to it the groove between α - and β -subunit formerly identified as the binding determinant (244).

The resulting complex suggests that the extracellular domain of the receptor is “J” or “U” shaped, lying on and making several contacts with the transmembrane domain. The hormone interacts only with the curved portion of the U and loosely occupies the space between the arms without necessarily making other contacts with the receptor. In this way the sugar moiety attached to α Asn52, necessary for signal transduction, is in close proximity to the N terminus of the extracellular domain, at the end of one U arm. Signal

transduction ensues from the steric effect of the oligosaccharide of α Asn52, which widens the distance between the two arms of the U. Since the extracellular domain has contact with the transmembrane domain at several points, broadening its inner space results in a conformational change of the helical arrangement: the receptor is now "isomerized" in its activated state (250). An important limitation of this model is that the C- and N-terminal ends of the extracellular domain were modeled as LRR, although such structural motifs cannot be easily identified in exon 1 and in exon 9 (214). However, the model is fully consistent with the concept of a unique, high-energy contact point between hormone and receptor (251) and explains and integrates knowledge on the role of carbohydrates on gonadotropin action.

Another model of the extracellular domain was obtained by computer-aided prediction of the tridimensional structure considering only seven LRR in the FSH receptor (214). Despite very low sequence similarity to ribonuclease inhibitor, the structure of the extracellular domain of gonadotropin receptors appears to be very similar, with the extracellular receptor domain shaped as a semibarrel extended over a third of a complete circle. The inner, concave surface of the extracellular domain is highly negatively charged, a feature suggesting that the positively charged gonadotropin would bind to it. Also considering shape complementarity, the model suggests that the long axis of the hormone would be positioned along the longitudinal barrel axis with both extremities extending out of the barrel. The carbohydrate moieties of the receptor would be directed away from the outer surface of the extracellular domain and should not participate in hormone binding. In this model, receptor activation results from interaction of the hormone end including L2 β , L1 α , and L3 α with the extracellular domain, whereas the role of glycosylation of hormone position α 52 would be to help position hCG in a favorable orientation for signal transduction (214).

In the model of Remy *et al.* (215) it is the second extracellular loop, connecting transmembrane domains 4 and 5, that triggers the conformational change necessary for signal transduction. This loop would interact with the lower pole of hCG, including L1 α and L3 α , which would then be responsible for receptor activation. Therefore, the role of the extracellular domain is to prevent the access of any glycoprotein hormone to the loop that can be activated by sequences of the common α -subunit.

VIII. Signal Transduction and Postreceptor Events

A. Properties of FSH receptor binding

The binding of FSH to its receptor is a process that, *in vitro*, is highly dependent on temperature and salt concentration and becomes slowly nonreversible at 37 C (186, 188, 252). Interestingly, only a few minutes are required to attain steady state receptor activation, as measured by cAMP production in Sertoli cells, whereas the development of high-affinity receptor binding requires several hours (187). Removal of bound FSH from granulosa cell membrane preparations requires strong acidic treatment (253), and binding is fully reversible only when hormone-receptor as-

sociation occurs at 4 C (186). The molecular mechanism of the slow formation of an irreversible interaction between FSH and its receptor is unknown. The factors proposed to participate in the stabilization of hormone-receptor binding include transglutaminase activity of the Sertoli cells (254–256), calcium (257–259), and interchange of disulfide bonds (260–263). However, the function of these factors has not been proven convincingly, and their role in FSH action remains an open question. The formation of peptide bonds between hormone and receptor is unlikely, since bound FSH can be dissociated at low pH (264). According to a model recently proposed, the formation of high-affinity hormone-receptor complexes might involve the generation of multimeric complexes (188).

Whether the stabilization of FSH receptor occurs *in vivo* is not known, but, in such a case, it could be an important step for hormone-receptor internalization. It has been shown that the gonadotropin LH reaches its target cells in the testis after crossing the endothelium via LH-mediated transcytosis and release of the hormone in the interstitium (48, 49). A similar mechanism of transportation has been recently proposed for the FSH receptor (44).

B. Role of receptor glycosylation and "cryptic" receptors

One interesting issue is the functional role of glycosylation of gonadotropin receptors. The FSH receptor possesses three (rat) or four (human) potential glycosylation sites in the extracellular domain. Earlier experiments based on glycosidase treatment of gonadal preparations showed that desialylation increases receptor binding of labeled gonadotropins. Moreover, not all the FSH receptors expressed at the surface of Sertoli and granulosa cells are available for binding. Enzymatic deglycosylation of plasma membranes unmasks cryptic binding sites in testicular preparations from calves (265) and monkeys (30) and in porcine granulosa cells (266). Unmasking of functional FSH receptors in porcine granulosa cells was also obtained by pretreatment with FSH, which increased the number of binding sites while decreasing cell responsiveness in terms of cAMP production (253). However, neuraminidase was not able to mimic the unmasking effect of FSH when membrane preparations were used instead of whole granulosa cells (253), and the functional meaning of cryptic receptors remains unclear. The concept that local enzymatic activities in the gonads could potentially modulate hormone action by unmasking receptors (266) remains highly speculative.

Experiments employing site-directed mutagenesis have shown that at least two of the three potential glycosylation sites of the rat FSH receptor are actually N-linked glycosylated (85). Removal of N-linked glycosylation sites did not alter the binding affinity of the mutated receptor, but binding was lost when both glycosylation sites were mutagenized or when glycosylation of the nascent receptor was totally prevented by tunicamycin. In contrast, exhaustive deglycosylation of mature receptors did not impair binding properties, suggesting that carbohydrates are essential for receptor folding and transportation to the cell surface, but not for hormone binding (85). Moreover, deglycosylation of the calf

testis receptor did not affect functional coupling to G_s protein (201). Similarly, carbohydrate residues are not involved in hormone binding and signal transduction in the rat ovarian LH receptor (267). No unmasking of cryptic receptors has been described using recombinant cell lines.

C. Signal transduction

1. Protein kinase A (PKA)-regulated pathway. It has been known for a long time that FSH induces an increase of intracellular cAMP levels in Sertoli and granulosa cells (reviewed in Ref. 58). The sequence of events after the interaction of FSH with its receptor is shown schematically in Fig. 7. After interaction with the hormone, the receptor becomes coupled to G_s , which, in turn, stimulates adenylyl cyclase and production of cAMP (268). A key step is the association of the receptor to G_s (269) that can occur only when the receptor is in an "active" state. This implies that the receptor exists in an equilibrium between two forms, inactive and active, the second state resulting from a conformational change of the inactive form. The interaction between hormone and receptor determines receptor isomerization to the active form and initiates the cascade of events collectively indicated as signal transduction (250). This mechanism is common to many G protein-coupled receptors, and it is now known that the hormone is not the only possible activator. Site-directed mutagenesis studies and the natural occurrence of "activating" mutations have demonstrated that G protein-coupled receptors are normally constrained in a resting state, and any events disturbing this state (e.g., interaction with the hormone or mutations resulting in conformational changes) trigger cAMP production and signal transduction (5, 8, 10, 250, 270).

In the case of FSH, the increase of intracellular cAMP in granulosa or Sertoli cells activates PKA, which phosphorylates structural proteins, enzymes, and transcriptional activators. Among the latter, the family of cAMP responsive elements (CRE) binding proteins (CREBs) and modulators (CREMs) is currently being intensively investigated (reviewed in Refs. 271, 272). Depending on the endocrine status and stage of gamete maturation, various alternatively spliced isoforms of CREM and CREB are preferentially expressed, leading to activation or repression of genes carrying a CRE in their promoter region (110, 272–275). In the testis, spermatogenic cells and Sertoli cells express several isoforms of CREB and CREM (271). In CREM τ knockout mice a spermatogenic arrest at the spermatid level is observed, indicating the importance of this signaling cascade in spermatogenesis (276, 277).

FSH induces rapid phosphorylation of CREB in Sertoli cells, which, as a result, stimulates cAMP responsive genes and autoregulates its own expression (278). The CREM isoform ICER, a putative repressor of FSH receptor expression, seems to be involved in the down-regulation of CREB expression in Sertoli cells. Together with the stage-specific expression of the FSH receptor, this may explain the oscillating cAMP-mediated CREB expression during the spermatogenic cycle (271).

Whether the FSH receptor is also linked to G_i is not com-

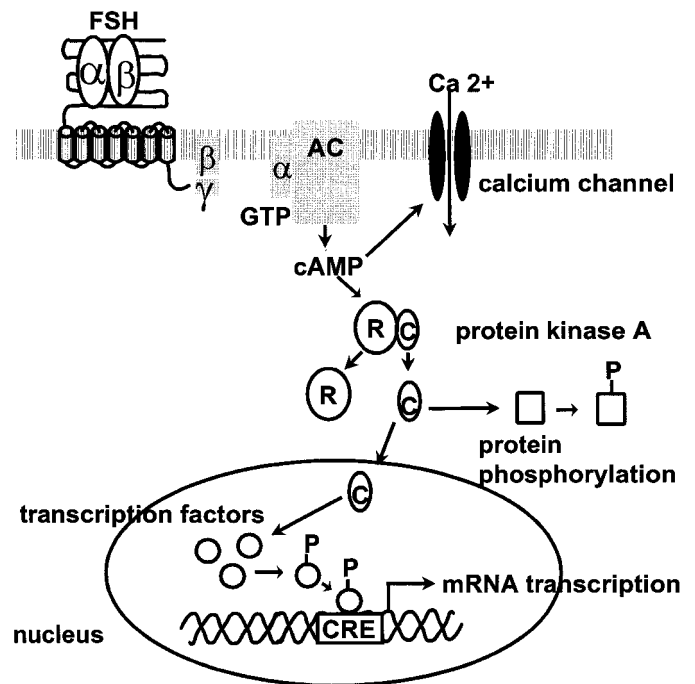


FIG. 7. Model of signal transductional pathways of the FSH receptor. Upon binding of FSH to the FSH receptor, the $G_{s\alpha}$ subunit dissociates. Together with GTP, this complex directly activates adenylyl cyclase, thereby leading to cAMP synthesis. PKA is activated by cAMP, which causes the dissociation of the catalytic subunit (C) from the regulatory subunit (R). The active catalytic site can activate proteins by phosphorylation. Conversely, the production of cAMP leads to an intracellular rise of Ca^{2+} , presumably due to the gating of calcium channels. In the nucleus the catalytic subunit of the PKA can phosphorylate transcription factors such as CREB and CREM, which then bind to CREs preceding certain genes. Finally, mRNA synthesis of primary response genes of FSH action starts.

pletely clear. In Sertoli cells and in isolated seminiferous tubules, treatment with pertussis toxin, an agent capable of removing tonical inhibitory effects of G_i , increases FSH-induced aromatase activity (279, 280) and suppresses the FSH-dependent increase of intracytoplasmic calcium (281). The data suggest that the inhibitory activity of G_i may negatively modulate the PKA pathway of signal transduction.

2. Protein kinase C (PKC)-regulated pathway. Sertoli cells possess the PKC pathway, but the FSH effect on it is unclear. The exposure of Sertoli cells to agents stimulating the PKC pathway has been shown to inhibit FSH-dependent cAMP production (282, 283), while FSH itself does not activate (284) or even inhibits (282) the phosphatidylinositol pathway. In contrast, the rat FSH receptor permanently overexpressed in the human kidney 293 cell line couples to the PKC pathway, and pharmacological concentrations of FSH (100 ng/ml or more) elicit a dose-dependent increase of inositol phosphates (177). However, overexpression of recombinant G protein-coupled receptors in cell lines may result in stimulation of uncommon signal transduction pathways (200), whereas such high FSH doses have not been investigated in Sertoli cells. Studies with chimeric human LH/FSH receptors in 293 cells showed that the capability of inducing inositol phosphate release is weak in the FSH receptor and resides in the

C-terminal third in the LH receptor (285). Overall, the impact of dual receptor coupling *in vivo* remains unclear.

3. *Calcium*. FSH increases intracellular calcium concentrations in Sertoli cells (58, 279, 286) and granulosa cells (287) and induces calcium uptake by FSH receptor-containing proteoliposomes (288). There is little doubt that the main effect of FSH is a receptor-mediated stimulation of extracellular calcium entry into the cell via both voltage-gated and voltage-independent, ligand-mediated calcium channels (286, 288). However, the suggestion that the FSH receptor itself may function as a ligand-gated calcium channel (279, 288) was not confirmed using recombinant FSH receptors (175). It is likely that FSH exerts its effect on calcium by stimulating other calcium channels preexisting on granulosa and Sertoli cells, *e.g.*, through mechanisms involving changes in membrane potentials (289). The postulated role of hydrolysis products of internalized FSH-receptor complexes as inducers of channel formation at the cell membrane has not been confirmed (290, 291).

There is no agreement as to whether the effects of FSH on calcium are mediated by cAMP and the PKA signal transduction pathway (279, 281, 286, 292, 293), and global interpretation of the data is rendered complex by the differences in the experimental approaches employed. For example, 1 mM of (Bu)₂cAMP was unable to stimulate uptake of ⁴⁵Ca²⁺ by Sertoli cells maintained in culture for 24 h (279). When the calcium entry into Sertoli cells was measured using the fluorescent dye Fura2-AM over a period of 4 min, 1 mM (Bu)₂cAMP stimulated cytosolic calcium levels, mimicking the effects of FSH (286). The same method applied to individual granulosa cells suggested that FSH effects could be partially due to cAMP, and whereas FSH was unable to increase intracellular calcium in the presence of extracellular calcium chelators, 8-Br-cAMP and forskolin retained the property of inducing a calcium signal (287). Successive experiments suggested that FSH effects on granulosa cells involved not only PKA activation and that cAMP might increase intracellular calcium by mobilizing it from internal stores (292), an effect, however, independent of PKC. Further experiments on Sertoli cells indicated that FSH action on calcium might be mediated partially by cAMP (281) and partially by other mechanisms (294). Using individual Sertoli cells and the fluorescent dye method, it was shown that, in the presence of extracellular calcium, FSH stimulates the uptake of fixed amounts of calcium. The effect was not dose-dependent, and increasing the hormone concentrations augmented the number of cells actively incorporating calcium. This effect could be partially mimicked by cAMP analogs and PKA activators but also occurred partially in the absence of extracellular calcium (293).

Collectively, these data indicate that the FSH effect on extracellular calcium entry partially involves the receptor-mediated PKA pathway. Other effects on intracellular calcium mobilization probably do not involve an FSH receptor-mediated mechanism. However, the role of intracellular calcium response in Sertoli and granulosa cells is unknown. Speculatively, calcium might amplify or modulate the cAMP signal transduction (286).

D. Receptor desensitization

Continuous exposure to the hormone leads to a decreased response, a process called desensitization. In the absence of FSH, granulosa cells *in vitro* rapidly lose FSH binding sites (295), but incubation in the presence of FSH also results in a dose- and time-dependent loss of receptors referred to as down-regulation (113). The incubation of Sertoli cells in the presence of FSH desensitizes adenylyl cyclase with a decrease of cAMP production preceding the FSH receptor loss (296). *In vivo*, an increase of testicular FSH receptors follows hemicastration and the compensatory rise of circulating FSH (297), but receptor depletion ensues from gonadotropin administration to intact animals (61). Obviously, target cells protect themselves from overstimulation by modulating the availability and functionality of FSH receptors, depending on the presence or absence of the hormone. This occurs through cyclic increases and attenuation of responsivity involving multiple regulatory mechanisms, eventually resulting in refractoriness to a permanent stimulation (298). Desensitization consists of a succession of events which, on a theoretical level, can be distinguished into rapid and slow processes, leading to reduction of receptor function (uncoupling) and number (down-regulation), respectively.

1. *Decrease of receptor function (uncoupling)*. Apart from an increased phosphodiesterase activity and consequent cAMP degradation (299, 300), an early event after receptor stimulation is agonist-induced receptor desensitization, due to uncoupling of the FSH receptor from G_s (300–302). Uncoupling occurs through enzymatic phosphorylation of the C-terminal, intracellular domain of G protein-coupled receptors and may be due to receptor-specific kinases or to effector kinases typical of the receptor system such as PKA or PKC. Usually, Ser and/or Thr residues can be phosphorylated, an event that promotes binding to the phosphorylated receptor of inhibitor proteins called arrestins that ultimately interrupt interaction between the receptor and the G protein. This process has been better characterized for the β-adrenergic receptor (Ref. 303; reviewed in Ref. 304), and the involvement of arrestins in uncoupling of glycoprotein hormone receptors is not yet characterized.

Receptor uncoupling is presently studied not in the natural, receptor-bearing cells but, more practically, in cell lines of various origins overexpressing the receptor under investigation. It is clear, however, that complex processes such as receptor uncoupling depend on the receptor as well as on the enzymatic equipment and global intracellular apparatus of the host cell, which do not necessarily correspond to the natural, *in vivo* situation. On the other hand, studies on post-translational receptor modification are difficult in primary cell cultures or transformed gonadal cell lines for low receptor expression. The effects of gonadotropin receptor phosphorylation have been recently reviewed (305). Briefly, as for the FSH receptor, studies employing the human 293 embryonic kidney cell line permanently transfected with the rat FSH receptor cDNA have shown that phosphorylation is induced by FSH and phorbol esters and does not involve PKA (177). In this cell line, receptor phosphorylation was evident within a few minutes, but was sustained and better detected during incubations lasting up to 1 h (178). The dose

of FSH required (EC_{50} , 30 ng/ml) was well in the range typical for induction of FSH-dependent events in Sertoli cells, indicating its physiological relevance (178). Furthermore, it was determined that phosphorylation occurs both on Ser and Thr residues and that residues involved in this process are, most probably, located upstream of aa position 635 (178). Although the partial involvement of PKC in the phosphorylation of the FSH receptor is possible (177), FSH action seems to be mediated rather by a specific receptor kinase (177, 178, 305, 306).

2. Decrease of receptor number (down-regulation). As a part of the desensitization process, receptor numbers decrease through internalization and sequestration of hormone-receptor complexes in the lysosomes (307–310) or reduced receptor protein synthesis as a result of both decreased transcription and/or reduced mRNA half-life. Receptor sequestration is already evident after 1–4 h and precedes the decrease of protein receptor synthesis, a down-regulation mechanism requiring about 24 h in cultured Sertoli cells (145). Within 4 h FSH also induced a decrease in FSH receptor mRNA due to a cAMP-dependent, posttranscriptional mechanism (145). Over longer time periods, FSH causes a dose- and time-dependent decrease of functional FSH-binding sites in porcine granulosa cells maintained in culture for up to 6 days. However, at the end of this time span, the FSH receptor mRNA increases, an effect mimicked by cholera toxin (113).

A mechanism whereby FSH decreases the expression of its own receptor might be the stimulation of ICER expression in Sertoli cells (110). ICER is a CREM isoform that blocks transcription of genes by binding to a CRE-like sequence in their regulatory region. In cultured Sertoli cells, FSH stimulates maximal ICER expression after 4 h, and the ICER protein levels remain elevated up to 24 h. ICER could thereby repress FSH receptor transcription (110). However, as discussed previously, it should be considered that, unlike the rat FSH receptor gene, both the mouse and human FSH receptor genes lack CRE-like elements in their promoter region (106, 107), and the effects of ICER on FSH receptor mRNA in these species needs confirmation.

E. Postreceptor events

As mentioned above, FSH receptor activation and cAMP production induce activation of PKA, which, in turn, phosphorylates Ser and/or Thr residues of cellular proteins. This process results in a number of postreceptor events, overall constituting the biological response of the target cell to FSH. The current view assumes that many intracellular processes originate from phosphorylation of a few, very specific, proteins, such as metabolic enzymes and transcription factors (CREB). Therefore, the intracellular events after receptor activation are, primarily, regulation of metabolic function and induction/suppression of the transcription of genes bearing a CRE in their promoter region or other *cis*-elements responsive to cAMP. When the transcription of FSH-inducible genes does not require synthesis of intermediate proteins, it is considered to be an immediate, direct effect of receptor activation and leads to the regulated expression of so-called primary response genes. Among them, *c-fos* and *junB* have

been known for long time and function as *trans*-acting factors controlling the expression of further genes and thereby mediate the secondary metabolic and trophic actions of FSH (311, 312). Other primary response genes have been identified recently and include potential RNA-binding proteins (regulation of RNA translocation and splicing), a mitochondrial transcript, other transcription regulators, and a possible antiproliferative factor (313). Further primary response events induced rapidly by FSH are the induction of the CREM isoform ICER (110) and the phosphorylation of CREB (271). Interestingly, cyclin D2, a cell cycle-associated factor, has been recently shown to be FSH-dependent and to be involved in the genesis of ovarian and testicular germ cell tumors (314). The human homolog of another FSH primary response gene named LRPR1 (leucine-rich primary response gene 1) and first isolated in the rat (315) has been mapped on chromosome X (316); however, the function of this cytoplasmic protein remains to be shown. In Sertoli cells, FSH also regulates the expression of the androgen receptor (317), DAX-1 (318), stem cell factor (319), and inhibin- α (320) mRNA. Most of these effects, however, cannot be allocated exclusively to FSH and can be induced by other factors as well. A number of good articles have examined in detail the biological actions of FSH in the testis and the ovary (2, 4, 156, 321–323), a topic beyond the scope of this review.

IX. Inhibitors and Modulators of the FSH Receptor

The presence of inhibitors of FSH binding in tissue extracts and biological fluids was already noted during the first experiments with rat FSH receptor preparations (17). Invariably, attempts to purify the FSH receptor biochemically were accompanied by evidence of substances interfering with FSH binding to its receptor. In the following years the concept of specific inhibitors of FSH binding evolved parallel to the characterization of the FSH receptor and led to the isolation and partial characterization of low and high molecular weight substances in various tissue preparations from different animal species. The common feature of these substances is that they compete and displace labeled FSH from its receptor in binding assays (17, 324–330). Some binding inhibitors are capable of antagonizing FSH activity in *in vitro* bioassays (331, 332). Intriguingly, some of them show FSH-agonistic activity (331, 333) and even FSH-like immunoreactivity (333, 334). The description of these substances gave support to the idea that local and/or systemic modulators could regulate FSH binding to the receptor and bioactivity in the gonads (335, 336). It should be noticed, however, that most of these substances have never been purified to homogeneity or definitely identified at the aa level. The evidence is scarce that they have any role *in vivo*.

FSH-binding inhibitors have been described in the bovine ovary (325, 327, 336, 337) and follicular fluid (328, 330–332, 334, 338–341). Recently, it was found that human follicular fluid collected from women undergoing superovulation for *in vitro* fertilization procedures contains a high molecular weight binding inhibitor (342) which, curiously, possesses FSH agonistic activity and immunoreactivity (333), but is larger than FSH and has a different aa composition (343).

Given the source of the material, *i.e.*, follicular fluid from women treated with FSH, the biological and immunological properties of this substance are puzzling, especially considering that the same fluid might contain FSH receptor-related soluble proteins (207). However, one candidate for the FSH-modulating activity in follicular fluid is the α -inhibin precursor (344).

The testis (324, 329, 345–348) and serum (326, 349, 350) are other sources of putative FSH-binding modulators, but their exact chemical nature was never characterized. Fractions obtained from isoelectrofocusing separation of crude human serum contain both factors with FSH-like bioactivity and factors that apparently inhibit the bioactivity of serum immunoreactive FSH (184). The presence in serum of factors with stimulatory and inhibitory activity on granulosa and Sertoli cells *in vitro* is well known (351–354) and can lead to major interference in *in vitro* bioassays (355). In some bioassay systems, serum samples need to be extracted (356) or heat-inactivated (183) before incubation with the cells to remove the undesired inhibitory activity. Although important for the practical purposes of *in vitro* bioassays, the serum FSH inhibitors seem to be aspecific, and there is no convincing evidence of any physiological role *in vivo*. Using a radioligand receptor assay the presence of low molecular weight binding inhibitors was detected in serum from patients with POF (357, 358). However, whether these substances are particular to this clinical condition has not been demonstrated.

Circulating FSH/FSH receptor antibodies have been reported sporadically in patients treated with exogenous gonadotropins (reviewed in Ref. 335), in two women with hypergonadotropic amenorrhoea, and in one hypogonadal man (359, 360). On the other hand, neutralization of FSH action and infertility was achieved by immunizing monkeys against FSH (361–363). Therefore, antibodies against the FSH receptor might similarly impair FSH action and lead to gonadotropin resistance and infertility. Recently the association between anti-FSH receptor antibodies and other circulating autoantibodies has been suggested in women with resistant ovary syndrome (364). However, in infertile men with various types of circulating autoantibodies, we were unable to detect Igs interfering with FSH action on rat Sertoli cells *in vitro* (365). Accordingly, the reanalysis of the occurrence of specific receptor antibodies in POF, using a homologous system based on recombinant receptors, showed no evidence of Igs interfering with FSH or LH action (186). In conclusion, the natural occurrence of anti-FSH receptor antibodies must be extremely rare. The development of recombinant receptor systems will allow the thorough investigation of serum FSH receptor inhibitors in various clinical conditions (194).

X. Naturally Occurring Mutations of the FSH Receptor

Several mutations of the TSH and LH receptor have been recently identified and considered to be the cause of specific diseases (10, 12, 91). Since altered LH-LH receptor interaction invariably results in altered testosterone production, mutations in the LH receptor have a strong impact on the male phenotype. Activating mutations of the LH receptor lead to

male-limited pseudoprecocious puberty (Refs. 366 and 367; reviewed in Ref. 368), whereas inactivating mutations result in male pseudohermaphroditism (369). For the TSH receptor, somatic activating mutations are supposed to play a role in the development of hyperfunctioning thyroid adenomas, due to their mitogenic activity on thyroid follicular cells (370). On the other hand, inactivating mutations are associated with some rare forms of congenital hyper- and hypothyroidism (371, 372).

Unlike the cognate LH and TSH receptors, mutations of the FSH receptor have not been easy to identify. This is partly due to our incomplete knowledge of the molecular action of FSH on the gonads and partly to the virtual absence of diseases characterized by isolated deficiency or hypersecretion of FSH. Therefore, the phenotypes related to FSH ineffectiveness or hyperstimulation might remain overlooked. Tentatively, some help in identifying the possible phenotypes related to inactivating and activating mutations of the FSH receptor can be obtained from mutations of the FSH β -subunit and of G_s , respectively (373, 374).

A. Inactivating mutations

The phenotype associated with a homozygous nonsense mutation of the FSH β -subunit gene, leading to premature termination of the protein chain, is primary amenorrhoea (373). A corresponding clinical picture in the male has not yet been described. If FSH is necessary for spermatogenesis, one could expect that elimination of FSH action would lead to male infertility in the presence of normal androgenization. This knowledge prompted the search for inactivating mutations of the FSH receptor in primary amenorrhoea and in infertile men.

Pure ovarian dysgenesis is a disease characterized by normal karyotype, highly elevated gonadotropins, and streaky gonads associated with primary amenorrhoea. A large genetic survey of pure ovarian dysgenesis in Finland led to the identification of several families showing an autosomal recessive pattern of inheritance (375). By genetic linkage analysis, the locus segregating with the disease was mapped to chromosome 2p. This position corresponds to the chromosomal localization of both gonadotropin receptors (93, 95). Since the male siblings in these families did not show any particular phenotype and were normally androgenized, indicating normal LH action, mutations in the FSH receptor were considered as a probable cause for the disease. By screening the FSH receptor gene in the affected families, a mutation was identified showing a transition from Ala to Val at position 189 in the extracellular receptor domain. This mutation was found to be homozygous in all affected females and segregated perfectly with the disease phenotype. Functional studies using a mouse Sertoli cell line revealed a nearly complete lack of cAMP production by the mutated receptor upon FSH stimulation. FSH-binding capacity of the mutated receptor was greatly diminished, but ligand-binding affinity was apparently normal (7). The clinical features of these patients were analyzed subsequently, and both transvaginal sonography and ovarian histology revealed the occurrence of primordial ovarian follicles (376). Since follicles were not demonstrated in patients with ovarian dysgenesis and nor-

mal FSH receptor, the phenotype related to the Ala 189 Val mutation suggests the possibility of some residual receptor activity (376).

The comparison of the aa sequence of FSH receptors from different species and other glycoprotein hormone receptors yields interesting insights into the possible role of this *per se* minor Ala-to-Val aa substitution in the receptor. A stretch of 5 aa, including Ala at position 189, is perfectly conserved within all glycoprotein hormone receptors and within the FSH receptor from different species (Fig. 2). This sequence contains a N-linked glycosylation site, underlining the functional importance of this region. Studies on the glycosylation pattern in the rat FSH receptor indicated that this glycosylation site is important for the proper folding of the protein (85). Therefore, a mutation within this region could affect conformational integrity. Since the binding affinity in the mutated receptor was normal, the mutation presumably disturbs the trafficking of the receptor to the membrane.

In a healthy woman, we have recently identified a heterozygous point mutation directly involving the same glycosylation site, changing Asn to Ile at position 191 (Fig. 2). Functional studies in COS-7 cells, comparing cAMP production after FSH stimulation by mutated and wild type receptor, revealed a pattern similar to that obtained in the presence of the Ala 189 Val mutation. Stimulation with FSH of cells transfected with the mutated receptor could induce only minimal cAMP production, compared with a marked cAMP increase in cells transfected with the wild type receptor (Fig. 8). This finding confirms the crucial importance of this receptor region. Most importantly, this woman recently became pregnant, indicating that heterozygous inactivating mutations of the FSH receptor are fully compatible with normal fertility.

Inadequacy of the FSH receptor has been repeatedly reported to be involved in idiopathic infertility (35, 36). After resolution of the genomic structure of the FSH receptor gene (99), we undertook the screening of a large collective of male patients with idiopathic infertility for mutations of the FSH receptor. Unfortunately, no mutations could be identified, even when a very selected group of azoospermic men with histologically documented Sertoli-cell-only syndrome was investigated (193). The previous descriptions, based exclusively on FSH-binding studies, were probably vitiated by methodological limitations, such as the possible presence of interfering substances in the binding assay (see Section IX). We have to conclude, therefore, that inactivating mutations of the FSH receptor are not a major cause of infertility in the male.

Five male sibs of the Finnish females with ovarian dysgenesis found to be homozygous for the Ala 189 Val-inactivating mutation were shown recently to have variable degrees of spermatogenic failure. However, none of them was azoospermic, only one was infertile, and two of them had fathered children (377). Together with the recent finding of normal fertility in FSH β knockout male transgenic mice (378), this finding might indicate that FSH is not absolutely essential for male fertility. However, the possibility of some residual FSH receptor function in the Finnish men cannot be excluded (376). Moreover, the phenotype of the lack of FSH function in males, both men and mice, is characterized by a

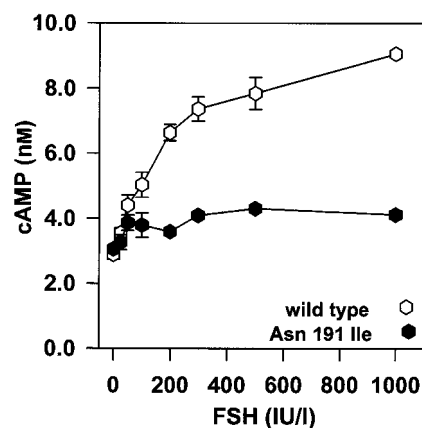


FIG. 8. Inactivation of the FSH receptor by the Asn191Ile mutation. cAMP production by COS-7 cells transiently transfected with constant DNA concentrations of wild-type (□) or Asn191Ile (■) mutated FSH receptor constructs and stimulated with increasing doses of human FSH. Results are expressed as the mean \pm SEM of two individual experiments performed in duplicate. [Reprinted from J. Gromoll *et al.*: *Mol Cell Endocrinol* 125:177–182, 1996 (394) with kind permission from Elsevier Science Ireland Ltd., Bay 15K, Shannon Industrial Estate, Co. Clare, Ireland.]

strong reduction of testicular volume and sperm output, confirming that FSH is essential for normal spermatogenesis.

Other pathological entities in which mutations of the FSH receptor might be a possible pathogenetic candidate are POF and the resistant ovary syndrome. As mentioned in Section IX, inhibitors and antibodies directed against the FSH receptor have been postulated to be involved in these diseases, but the problem might well reside in the receptor itself (379). Investigations in this direction are currently ongoing.

B. Activating mutations

Gonadal hyperactivation related to activating mutations of G_s in McCune-Albright's syndrome leads to gonocyte maturation and hypersecretion of steroid hormones with precocious puberty in both sexes (374). However, this clinical picture depends on the simultaneous hyperactivity of the effector system common to both gonadotropins, and the phenotype associated with an inborn, isolated constitutive activity of the FSH receptor is difficult to foresee. Before puberty, an isolated, chronic FSH-like stimulation probably has no consequence in females, where LH is necessary to produce the substrate for FSH-dependent aromatization. Similarly, in the male, puberty does not begin without testosterone (380). However, FSH action is necessary for gamete proliferation and maturation, and the effects of its chronic hyperactivity might become visible around puberty. Since patients with pituitary tumors hypersecreting FSH have been reported to have enlarged testicles (381), we investigated whether activating mutations of the FSH receptor could be found in patients with megalotestes (382). No mutations could be found until now, but it should be considered that, if megalotestis remains an isolated symptom in the presence of normal fertility, these cases could never reach clinical observation.

In the course of these studies we identified a patient who had puzzled us for several years. He had been hypophyse-

tomized because of a pituitary tumor and, under testosterone substitution therapy, was unexpectedly fertile in spite of undetectable serum gonadotropin levels. Testosterone treatment is given to hypophysectomized patients to maintain androgenization and in hypophysectomized patients does not prevent the drop in spermatogenesis resulting from gonadotropin withdrawal at least for some time. Therefore, if these patients wish to regain fertility, the treatment must include LH and FSH (1, 383). Surprisingly, this patient had ongoing normal spermatogenesis under testosterone alone and, despite the gonadotropin deficiency, he fathered three children. We suspected that his unexplained fertility might be sustained by an activating mutation of the FSH receptor. Screening of the FSH receptor gene led to the identification of a heterozygous mutation changing Asp to Gly at position 567, located in the third intracytoplasmic loop (8). Functional studies performed in transiently transfected COS-7 cells showed that the mutant receptor induced a slight but consistently reproducible increase in cAMP production independent of FSH stimulation, indicating constitutive activity (8, 384).

We have recently reanalyzed the functional properties of the mutated receptor using a line derived from mouse Sertoli cells (190). This cell line has lost the expression of the endogenous FSH receptor but, being derived from a cell type normally expressing the receptor, most probably possesses a signal transduction machinery more adequate for functional studies. Transfection of the mutant Asp567Gly receptor into this line resulted in 3-fold higher increased basal cAMP production in the absence of FSH than that observed in the presence of the wild type receptor, *i.e.*, a response twice as high as that obtained using COS-7 cells (8) (Fig. 9). Moreover, these experiments corroborate the constitutive activity of the mutated receptor. Although one might argue that this cell line allows a better expression of the mutated receptor compared with COS-7 cells, obviously the detection system based on a Sertoli cell-derived line improves the sensitivity of the functional assay. Future studies with constitutively active LH and FSH receptors in gonadal and nongonadal cell lines should give further insights into putative cell-specific elements involved in gonadotropin receptor signal transduction. Another parameter of FSH-like stimulation *in vivo* may be circulating inhibin. Using a new enzyme-linked immunosorbent assay specific for dimeric inhibin B (385), we found that the patient with the activating mutation of the FSH receptor had serum inhibin levels comparable to those found in a group of fertile men, a possible indirect index of FSH-like bioactivity *in vivo*.

The mutation is localized in a crucial region of the transmembrane domain, highly conserved in all glycoprotein hormone receptors, and within the FSH receptor of different species. The same Asp to Gly transition in the corresponding codon 619 of the TSH receptor and codon 564 of the LH receptor has been previously reported to lead to constitutive activation of these receptors and was found in patients with thyroid adenoma and pseudoprecocious puberty, respectively (12, 367).

The constitutive activity of the FSH receptor bearing the Asp 567 Gly mutation was recently challenged by a paper suggesting that transmembrane domains V and VI maintain

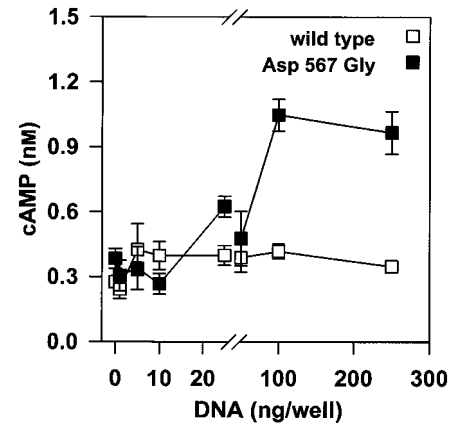


FIG. 9. Constitutive activation of the FSH receptor by the Asp567Gly mutation. Basal cAMP production by a Sertoli-cell line transiently transfected with increasing amounts of DNA (\square) wild-type or Asp567Gly (\blacksquare) mutated FSH receptor constructs. Results are expressed as the mean \pm SEM of two individual experiments performed in duplicate.

the FSH receptor in such a constrained state that the mutation has no appreciable effects on basal cAMP production when the receptor is expressed in 293 cells. On the contrary, the interaction between these domains in the LH receptor would be more permissive for constitutive activity of mutations in the third intracellular loop (199). This might explain the differences in the degree of constitutive activation observed for the same aa substitution in different receptors. In our experience there is no doubt that the activity of the mutated FSH receptor is lower compared with the other glycoprotein hormone receptors, but this has little relevance because, conceptually, "constitutive activity" does not depend on the "amount" of activation. Moreover, the results of Kudo *et al.* (199) were corrected by the amount of FSH binding to the mutated receptors assuming, without demonstrating it, that the correlation between FSH binding and cAMP production remains linear over varying ranges of FSH receptor numbers. Furthermore, the authors failed to comment on the significantly reduced effect of a saturating dose of FSH on the mutated FSH receptors. In summary, we believe that our *in vitro* data and the clinical evidence confirm that the Asp 567 Gly substitution indeed leads to constitutive activity of the FSH receptor.

To our knowledge, this remains the sole example of a naturally occurring activating mutation of the FSH receptor described so far. Since it was discovered because of the concomitance of hypophysectomy, it might well be that activating mutations of the FSH receptor remain asymptomatic in otherwise normal conditions. On the other hand, activating mutations of the FSH receptor have not yet been described in women either, leaving the possible phenotype still unrecognized in this gender as well. The effects of a ligand-independent activation of the FSH receptor on development and follicle maturation are speculative. Possible consequences of activating mutations might be POF, due to early ovarian exhaustion, or certain types of polycystosis. Moreover, the possible consequences of somatic, activating mutations include granulosa/Sertoli cell tumors, given the proliferation-

inducing properties of the gonadotropin. These possibilities will have to be investigated in the future.

The finding that an activating mutation in the FSH receptor gene in a hypophysectomized patient sustains spermatogenesis in the absence of gonadotropins provides an exceptional model for defining the role of FSH and testosterone in human spermatogenesis. This case suggests that FSH alone is sufficient for spermatogenesis in humans even in the absence of adequate concentrations of intratesticular testosterone. On the other hand, it is well known that both gonadotropins, FSH and LH, are necessary for fertility induction in hypogonadal patients (386, 387). These two pieces of apparently conflicting clinical evidence can be reconciled assuming that testosterone exerts a permissive role on the receptor-mediated FSH action, an effect not required if the FSH receptor is autonomously activated. Based on the findings that inactivating mutations do not lead to absolute infertility and that an activating mutation sustains spermatogenesis in the absence of gonadotropins, one might hypothesize that FSH alone and testosterone alone are both capable of maintaining spermatogenesis (388).

C. Allelic variants

The screening of the FSH receptor gene in patients with ovarian dysgenesis (7), in patients with POF (379), in women with hypogonadotropic hypogonadism (389), and in infertile men from our infertility clinic revealed that the FSH receptor is polymorphic in at least two sites. One polymorphic site is found in the extracellular domain at position 307, which can be occupied by either Ala or Thr, whereas position 680 in the intracellular domain can be occupied by either Asn or Ser (Fig. 10). To date nothing is known about the frequency and distribution of these allelic variants in the general population. Neither is it known whether these polymorphisms have any physiological impact on FSH binding and signal transduction. Polymorphisms have been reported in a variety of G protein-coupled receptors (390–392), and functional consequences have been shown for some of them. For example, hair and skin pigmentation is partially dependent on the functional diversity of allelic variants of the MSH receptor (390). Moreover, a TSH receptor variant showed enhanced sensitivity to TSH stimulation in CHO cells (392). Finally,

association between polymorphic receptors and pathological events has been described (393).

The significance of naturally occurring polymorphisms in the FSH receptor is still unknown (394). Since the functional consequences of the activating FSH receptor mutation are not impressive in transient transfection experiments, the occurrence of small changes in the functional properties of the receptor variants could require the use of a very specific and sensitive system. Furthermore, clinical studies about the presence and distribution of the receptor variants in the normal population will give some indications of the frequency of each polymorphism. Finally, it should be considered whether some receptor variant or combination of variants is related to a higher incidence of reproductive disorders.

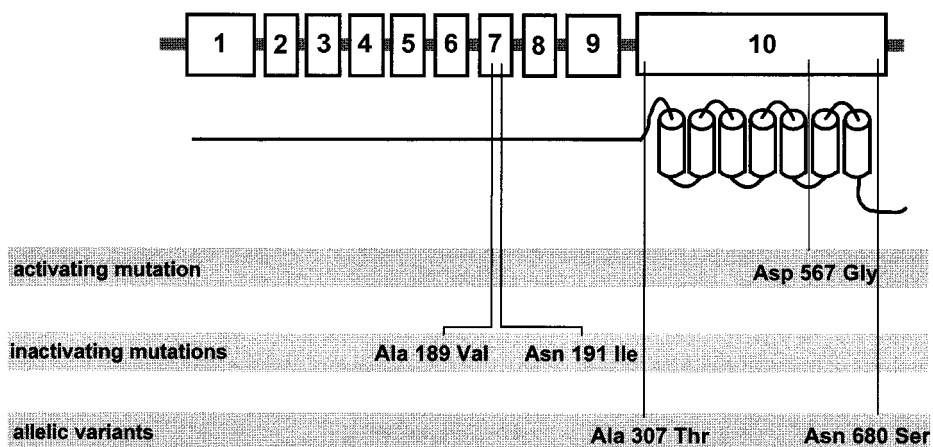
XI. Conclusions

From the discussion above it is clear that the FSH receptor is a unique member of the glycoprotein hormone receptor family. Although similarities like the genomic organization of the gene or the overall structure of the receptor protein are clearly visible, it displays remarkable specific characteristics.

Signal transduction of the FSH receptor seems to be nearly exclusively mediated by the PKA pathway, unlike the TSH and LH receptor in which dual signaling pathways have been demonstrated. The FSH receptor gene expression is highly gonad- and cell-specific, underlining its importance for oogenesis and spermatogenesis. The recent identification of inactivating and activating mutations enabled exciting new insights into FSH action. The essential role of FSH during ovarian development and normal oogenesis could be delineated, whereas the contentious issue of FSH action during spermatogenesis was further clarified. It now also becomes clear that the FSH receptor is polymorphic. To which extent these allelic variants have any physiological impact on reproduction still has to be shown.

Future studies should make use of DNA technology, thereby creating transgenic mice bearing constitutively activated FSH receptors or FSH receptor knock-out mice, which could then serve as models to further elucidate the role of FSH. In the case of the knock-out animals these would also

FIG. 10. Localization of the different naturally occurring mutations and allelic variants in the FSH receptor gene. For better understanding, a schematic protein structure of the FSH receptor is provided below the structural organization of the FSH receptor gene. The extracellular domain is indicated by a line, followed by the seven-transmembrane domains and the intracellular domain. The numbering of the different mutations includes the 17-residue signal peptide. [Adapted from J. Gromoll *et al.*: *Mol Cell Endocrinol* 125:177–182, 1996 (394) with kind permission from Elsevier Science Ireland Ltd., Bay 15K, Shannon Industrial Estate, Co. Clare, Ireland.]



have therapeutic dimensions for treating primary ovarian failure.

The central role of FSH in reproduction makes the FSH receptor a unique target for clinical purposes of fertility regulation. Based on functional binding studies, models of receptor activation, and signal transduction properties, the development of compounds with superagonist or antagonistic properties should now become possible.

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