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Review

The specificity of binding of glycoprotein hormones to their receptors

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Abstract. The glycoprotein hormone receptor family is peculiar because, in contrast to other G protein-coupled receptors, a large N-terminal extracellular ectodomain is responsible for hormone recognition. Hormone-receptor pairs have evolved in such a manner that a limited number of positions both at the 'seat-belt' domain of the hormone and the leucine-rich repeats of the receptor, play attractive and repulsive interactions for binding and specificity, respectively. Surprisingly, the constitutive activity of the receptor, mostly modulated by highly conserved amino acids within the heptahelical domain of the receptor (*i.e.*, outside the hormone binding region), also regulates effectiveness of hormone recognition by the extracellular part. In this review we analyze, at the molecular level, these important discriminating determinants for selective binding of glycoprotein hormones to their receptors, as well as natural mutations, observed in patients with gestational hyperthyroidism or ovarian hyperstimulation syndrome, that modify the selectivity of binding.

Keywords. Glycoprotein hormone receptors, hormone specificity, ovarian hyperstimulation syndrome, gestational hyperthyroidism.

Introduction

The glycoprotein hormone receptor (GpHR) family is formed by the thyroid-stimulating hormone receptor (TSHr), luteinizing hormone/chorionic gonadotropin receptor (LHCGr), and follicle-stimulating hormone receptor (FSHr) [1–5]. These three receptors respond to the glycoprotein hormones (GpHs) thyrotropin (TSH), lutropin (LH), chorionic gonadotropin (CG, with LH activity), and follitropin (FSH) to play key roles in the control of reproduction and metabolism. The GpHs are dimeric proteins of the cysteine-knot family, made of a common α-subunit and a specific β-subunit endowed with the functional and binding specificity [6–10]. The β-subunit of GpHs are encoded by paralogous genes displaying 40–43 % sequence identity [4], with 12 fully conserved disulfide-bonded cysteines. The 'seat-belt' segment of the β-subunit comprises the residues between Cys 10th and 12th Cys, categorized into the 'determinant loop' (between the 10th and 11th Cys) and the 'C-terminal loop' (between the 11th and 12th Cys) (Fig. 1A). The GpHRs are composed of a large N-terminal ectodomain (ECD),

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responsible for the high affinity and selective binding of the corresponding hormones [11–14], and a canonical portion made of seven transmembrane helices and a cytoplasmic α-helix parallel to the cell membrane, characteristic of the rhodopsin-like G protein-coupled receptor (GPCR) family [15–17], involved in transduction of the activation signal to the G protein(s) [4, 18]. The crystal structure of the human FSHr-FSH complex [14] has confirmed that the ECD of GpHRs belongs to the family of proteins with leucine-rich repeats (LRRs) [19] as was previously suggested by sequence analysis and homology modeling [20, 21]. The concave inner surface of the receptor (Fig. 1A) is an untwisted, non-inclined β-sheet formed by ten LRRs. Whereas the N-terminal portion of the β-sheet ($LRR_{1–7}$) is nearly flat, the C-terminal portion ($LRR_{7–10}$) has the horseshoe-like curvature of LRR proteins [14]. The crystal structure of part of the TSHr ECD in complex with a thyroid-stimulating autoantibody has recently been obtained [22]. Notably, both the structure of the ECD of TSHr and the receptor-binding arrangements of the autoantibody are very similar to those reported for the FSHr-FSH complex. Thus, despite the origins of these two ligands being very different, they show almost identical receptor-binding features [22]. The ECD of GpHRs also contains, downstream of the LRR region, a cysteine cluster domain of unknown structure (the hinge region), involved in receptor inhibition/activation [23–27] and containing sites for tyrosine sulfation important for hormone binding (see below).

Recent reports have indicated that single nucleotide substitutions of the TSH [28] or FSH [29–32] receptor genes are responsible for gestational hyperthyroidism or ovarian hyperstimulation syndrome [33], respectively. When tested *in vitro*, these mutant receptors displayed an abnormally high sensitivity to CG [34, 35], providing a sensible pathophysiological explanation to these diseases [36]. These natural mutations are located both at the LRRs of the ECD domain [28, 32], which directly contact the GpHs, and unexpectedly in the membrane heptahelical region [29–31]. The specificity of recognition of CG by TSHr and FSHr is, thus, of special relevance for coping with the elevated concentrations of CG during the first trimester of pregnancy [37] or in placental tumors [38]. In this review we analyze the important discriminating determinants for selective binding of GpHs to their receptors, using the crystal structure of the FSHr-FSH complex, natural or experimental gain of function mutants of GpHRs, and chimeras of the 'seat-belt' domain of GpHs.

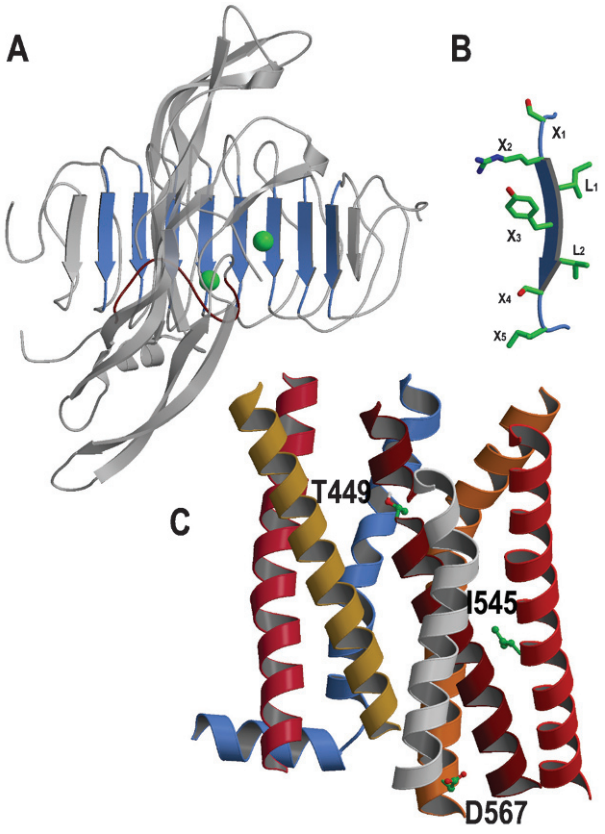

Figure 1. (*A*) General view of the follicle-stimulating hormone receptor (FSHr)-FSH crystal structure (PDB code 1XWD) [14]. The concave inner surface of the receptor, formed by ten leucine-rich repeats ($LRR_{2–9}$, shown in blue), contact the middle section of the hormone molecule, both the C-terminal segment of the α-subunit and the 'seat-belt' segment of the β-subunit (shown in red). The positions of the natural K183R and S128Y mutations in the ectodomain (ECD) of the thyroid-stimulating hormone receptor (TSHr) and FSHr, respectively, which affect human chorionic gonadotropin (hCG) specificity are shown (green spheres). (*B*) Each LRR is composed of the $X_1X_2LX_3LX_4X_5$ residues (where X is any amino acid, and L usually is Leu, Ile, or Val), forming the central $X_2LX_3LX_4$ a typical β-strand, while X_1 and X_5 are parts of the adjacent loops. (*C*) Molecular model of the transmembrane domain of the FSHr [34], constructed from the crystal structure of bovine rhodopsin [15]. The color code of the α-carbon ribbons is: transmembrane helix 1 (crimson), 2 (goldenrod), 3 (dark red), 4 (gray), 5 (red), 6 (orange), and 7 (blue), and Hx8 (blue). The positions of the constitutively active mutants T449I/A, I545T, and D567G/N in the FSHr gene found in patients with familial spontaneous ovarian hyperstimulation syndrome during pregnancy are shown. Very recently the crystal structure of the β_2-adrenergic receptor bound to the partial inverse agonist carazolol has been published [16, 17]. The structure of both rhodopsin and the β_2 receptor are similar at the transmembrane domain, thus, the constitutively active mutants would be positioned at similar positions in a β_2-based model of the FSHr.

The crystal structure of the FSHr-FSH complex

The FSHr binds FSH through the concave inner surface, in a manner that resembles a hand-clasp (Fig. 1A) [14]. A detailed description of this structure has been summarized elsewhere by Fan and Hendrickson [39, 40]. Here we only focus on the specificity

of hormone recognition. In general, the N-terminal parallel β-strands (LRR_{2-8}) of the receptor contact the middle section of the hormone molecule, both the C-terminal segment of the α-subunit and the 'seat-belt' segment of the β-subunit. As reported in the original publication the invariant acidic residues of GpHRs at the X_3 and X_5 positions (see Fig. 1 for notation) of LRR_6 (D150 and D153 in FSHr, E154 and D157 in LHCGr, E157 and D160 in TSHr) make salt bridges with the basic K91 and K51 residues, respectively, of the common α-subunit of GpHs (Fig. 2A, B). These interactions seem conserved among GpHs and, thus, the specificity of binding is guided by the 'seat-belt' portion of the β-subunit. The 'determinant loop' of the β-subunit contains a conserved acidic residue (D93 in FSH, D99 in CG, and D114 in TSH) to interact with a polar amino acid at the X_5 position of LRR_4 (K104 in FSHr, N107 in LHCGr, and N110 in TSHr) (Fig. 2A, B). Thus, primarily, the specific interactions between FSHr and FSH are: hydrophobic and ionic interactions between L55 (X_5 in LRR_2) and E76 (X_3 in LRR_3) and L99 and R97, respectively, both located at the 'C-terminal loop' of the hormone; and an electrostatic interaction between K179 (X_5 in LRR_7) with both S89 and D90 in the 'determinant loop' of the hormone (Fig. 2A, B). These positions in the ECD domain of GpHRs are 'hot spots' [41], *i.e.*, those with an important contribution in the selective binding of GpHs to their receptors (see below). The other numerous charged amino acids, at the FSHr-FSH interface, form networks of polar interactions: R52 (X_3 in LRR_2) with E50 (X_2 in LRR_2), K74 (X_2 in LRR_3) with E99 (X_2 in LRR_4), R101 (X_3 in LRR_4) with E103 (X_4 in LRR_4), R227 (X_5 in LRR_9) with D202 (X_5 in LRR_8), and R245 with D224 (X_3 in LRR_9) and E197 (X_2 in LRR_8). As a result the molecular electrostatic potential at the concave inner surface of the receptor is negatively charged (colored in red) between LRR_3 and LRR_6, and positively charged (colored in blue) at the bottom of LRR_7 and downstream from LRR_8 (Fig. 2C). Figure 2D shows the projection of the molecular electrostatic potential of the FSH hormone into the concave inner surface of the FSHr, to visualize the electrostatic interactions specific to each hormone-receptor couple. Obviously, the positive and negative areas of the FSHr (Fig. 2C) match with the reverse negative and positive areas, respectively, of the electrostatic projection of the FSH hormone (Fig. 2D).

It has been proposed that the mode of binding observed between FSH and the FSHr also pertains to the other members of the GpHR family [14]. This has been corroborated by the fact that ligands of different origin like FSH or a thyroid-stimulating autoantibody show almost identical receptor-binding features [22]. Thus, homology models of GpHRs in complex with different hormones [32], using the structure of the FSHr-FSH complex as a template, is a useful tool for understanding the key structural determinants of hormone specificity.

The homology model of the complex between LHCGr and CG

Substitution of the human CG (hCG) 'seat-belt' residues with their FSH counterparts increases binding to the FSHr and impedes binding to the LHCGr [9, 42]. It is the 'determinant-loop' of hCG that defines the specificity of binding since replacement of only the amino acids in this domain by the FSH counterparts decreases binding to the LHCGr, while an hCG chimera with the 'C-terminal loop' of FSH retains binding to the LHCGr [9, 42]. Interestingly, the 'determinant loop' of hCG has two basic residues (R94 and R95), while FSH has acidic residues in this part of the hormone (D88 and D90). Appropriately, X_5 of $LRR_{7,8,9}$ contain G183, E206, S231 in LHCGr and K179, D202, and R227 in FSHr (Fig. 2A, B), being the electrostatic surface in this area of the receptor negative in LHCGr and positive in FSHr (Fig. 2C). We propose that the hCG-specific R94 forms a salt bridge with E206 (X_5 in LRR_8), while R95 interacts with E154 (X_3 in LRR_6) (Fig. 2B). K179 of FSHr is a key determinant of the specificity against hCG because it is repulsive with R94 of hCG. In agreement the substitution of K179 of FSHr by Gly (the LHCGr counterpart) [13] or to other amino acids [43] gave a gain of sensitivity towards hCG. The conserved acidic D99 in the 'determinant loop' interacts with the polar N107 at the X_5 position of LRR_4 in LHCGr.

In contrast to the 'determinant loop' of hCG, the interactions of the 'C-terminal loop' of hCG and the LHCGr are similar to the interactions observed in the FSHr-FSH complex. The common negative E76 (FSHr) or E79 (LHCGr) side chain (X_3 in LRR_3) forms an ionic interaction with either R97 (FSH) or K104 (hCG) (Fig. 2A, B). However, the longer side chain of R97 (FSH) points towards bulk water in its interaction with E76 (FSHr), while the shorter side chain of K104 (hCG) points towards the core of the protein to interact with E79 (LHCGr), given that both amino acids are located one position apart in the 'C-terminal loop' (Fig. 2B). This change in orientation is necessary because in the FSHr-FSH complex a positive head group pointing towards the core of the protein would be repulsive with the positive FSHr-specific K104 at the X_5 position of LRR_4. Substitution of K104 of FSHr by Asn (the LHCGr counterpart) gave a gain of sensitivity towards hCG [13], revealing K104 of FSHr as a significant determinant of the specificity against hCG. Finally, D105 of hCG forms

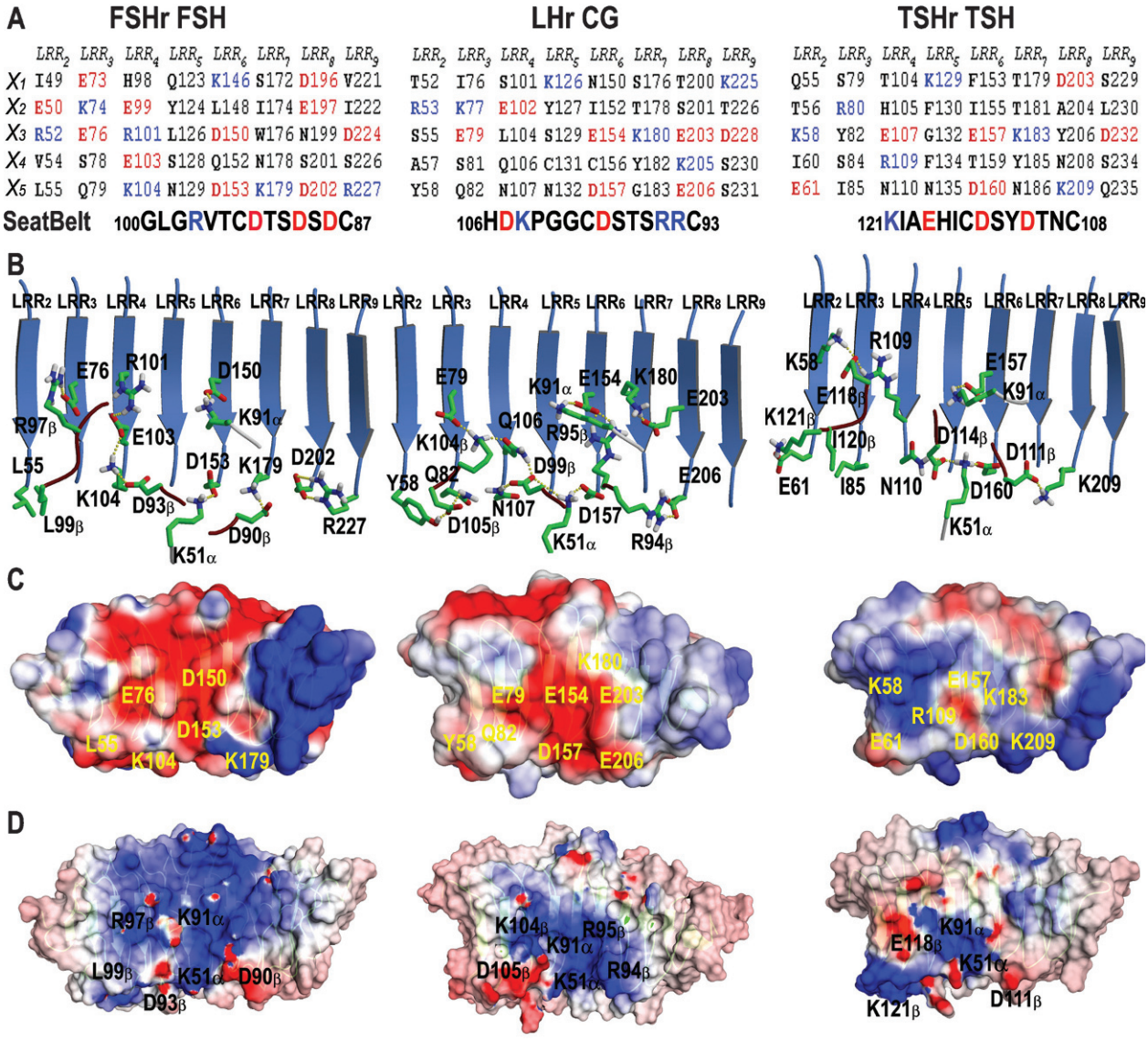

Figure 2. (*A*) Schematic representation of the X_{1-5} side chains in LRR_{2-9} of the glycoprotein hormone receptors (GpHRs) exposed to solvent and predicted to contact the hormones, together with the amino acid side chains of the seat-belt portion of the β-subunit of GpHs. Numbering of GpHRs and the β-subunit of the TSH starts from the first amino acid of the signal peptide, whereas in the case of hCG and FSH the signal sequence residues have been omitted. The notation of the amino acids is the same as in our previous original publications [4, 13, 32, 34, 35]. (*B*) Detailed view of the interaction between LRR_{2-9} of the receptor and the C-terminal segment of the α-subunit and the 'seat-belt' segment of the β-subunit of the hormone in the crystal FSHr-FSH structure and computer models of luteinizing hormone/chorionic gonadotropin receptor (LHCGr)-CG and TSHr-TSH complexes. (*C*) Molecular electrostatic potential at the concave inner surface of GpHRs. (*D*) The projection of the molecular electrostatic potential of the GpHs into the concave inner surface of the receptor to visualize the electrostatic interactions specific to each hormone-receptor couple.

hydrogen bond interactions with Y58 (X_5 in LRR_2) and Q82 (X_5 in LRR_3) in the LHCGr, replacing the hydrophobic interaction between L55 and L99 at the same positions in the FSHr-FSH complex (Fig. 2A, B).

The homology model of the complex between the TSHr and TSH

In TSH both the 'determinant loop' and the 'C-terminal loop' are important in TSHr binding. Substitution of the TSH residues of the 'determinant loop' or the 'C-terminal loop' or both at the same time by their hCG counterparts precludes TSH response [44]. The 'determinant loop' of TSH and hCG contain oppositely charged amino acids: the negative TSH-specific D111 and the positive hCG-specific R94 to form a salt bridge interaction with the positive K209 of TSHr and the negative E206 in LHCGr (X_5 of LRR_8), respectively. Thus, K209 in TSHr plays a key role in the repulsion against the R94 side chain of hCG. Accordingly, the K209E mutation in TSHr causes an

important gain of function towards hCG, while reducing strongly responsiveness to TSH [13]. Besides X_3 of $LRR_{6,7,8}$ also play a significant role in the selectivity of TSHr towards hCG through a repulsive mechanism. This central domain of the receptor recognizes the basic K91 residue of the common α-subunit through a conserved ionic interaction with the acidic amino acid at the X_3 position of LRR_6 (D150 in FSHr, E154 in LHCGr, E157 in TSHr). However, the contiguous amino acids at X_3 of $LRR_{7,8}$ are positive and negative in LHCGr (K180, E203), and positive and neutral in TSHr (K183, Y206). The electrostatic surface in this area of the receptor is positive in TSHr and negative in LHCGr (Fig. 2C). The hCG-specific R95 in the 'determinant loop' can form an ionic interaction with E154 in LHCGr, given that K180 is neutralized by its interaction with E203 (Fig. 2B). In contrast to LHCGr, E157 and K183 in TSHr are interacting [35] due to the absence of a negatively charged residues at position X_3 of LRR_8. Thus, the positive side chain of K183 is a key determinant of the specificity of the TSHR against hCG due to its repulsion with R95 of hCG. In agreement with this hypothesis, mutation of K183 to any amino acid [35] or adding a negative charge in X_3 of LRR_8 (the Y206E mutation) [13] increases the binding of hCG to TSHr. The conserved acidic D114 residue in the 'determinant loop' of TSH interacts with the polar N110 at the X_5 position of LRR_4 in TSHr, in a similar manner to the LHCGr-CG complex.

The type of interaction between LRR_{2-3} of TSHr and the 'C-terminal loop' of TSH is also divergent, relative to the LHCGr-hCG complex. X_3 of LRR_2 has K58 in TSHr, while X_3 of LRR_3 contains E79 in LHCGr. Thus, the electrostatics in this domain of the receptor are positive in TSHr, to form a salt bridge with the TSH-specific E118; and negative in LHCGr, to interact with the hCG-specific K104 of the 'C-terminal loop' (Fig. 2C, D). Accordingly, substitution of Y82 of TSHr by Glu (the LHCGr counterpart) increases the sensitivity towards hCG [13]. The importance of loss of basic and gain in acidic character in this region of the TSHr to increase sensitivity to hCG is also shown by K58 and R80 mutations in TSHr. When K58 was mutated to Ser, Ala or Asp; and R80 to Ala, a steady increase in sensitivity of the mutants to hCG was observed [13].

Finally, the 'C-terminal loop' of TSH possesses the basic K121 whereas hCG has the acidic D105 and FSH the hydrophobic L99, each one interacting with E61 in TSHr, Y58 in LHCGr, and L55 in FSHr (X_5 of LRR_2). In agreement, the substitution of E61 of TSHr by Tyr (the LHCGr counterpart) gave a gain of sensitivity towards hCG without changing the sensitivity to FSH, while E61A and E61L (the FSHr counterpart) TSHr substitutions provoke a gain of sensitivity towards FSH [13].

Transformation of TSHr or FSHr into LHCGr

Substitution of eight amino acids in TSHr for the corresponding LHCGr residues (K58S and E61Y in $X_{3,5}$ of LRR_1; R80K, Y82E, and I85Q in $X_{2,3,5}$ of LRR_2; and Y206E, N208K, and K209E in X_{3-5} of LRR_8), causes a gain of sensitivity towards hCG, matching that of the wild-type LHCGr [13]. Figure 3C shows the interaction between this mutant TSHr and hCG. Clearly, the E61Y and I85Q mutations facilitate the interaction with D105 of the 'C-terminal loop' of hCG; the Y82E mutation creates the acidic area in this part of the receptor to interact with K104 of the 'C-terminal loop'; the Y206E and N208K mutations adds in LRR_8 the charged amino acids observed in the LHCGr, facilitating the interaction of R95 of the 'determinant loop' with E157 and E206 of the mutant TSHr; and the K209E mutation facilitates the interaction with R94 of the 'determinant loop'. The electrostatic surface map of this mutant TSHr (Fig. 3C) differs considerably from wild-type TSHr (Fig. 2C) and resembles the map of wild-type LHCGr (Fig. 3B).

Similarly, substitution of two amino acids in FSHr for the LHCGr counterparts (K104N in X_5 of LRR_4, and K179G in X_5 of LRR_7) results in a mutant receptor displaying increased response to hCG [13]. The K104N mutation in FSHr removes the repulsion with K104 of the 'C-terminal loop' of hCG in its interactions with E76; whereas the K179G mutation facilitates the interaction with R94 of the 'determinant loop' (Fig. 3A). Clearly, there is a significant resemblance in the electrostatics at the LRR_{2-9} domain between this mutant FSHr (Fig. 3C) and wild-type LHCGr (Fig. 3B).

Co-evolution in the specificity of hormone recognition

The high sequence similarity between the β-subunit of GpHs and the GpHRs [4], which are encoded by paralogous genes, together with the above results illustrates how selective binding of GpHs to their respective receptors is the result of a co-evolutionary mechanism [9]. Hormone-receptor pairs have evolved in such a manner that certain 'hot spot' positions both at the 'seat-belt' domain of the hormone and the concave inner surface of the receptor play attractive and repulsive interactions for binding and specificity, respectively. The key important discriminating-determinants for selective binding are the following (Fig. 4). The X_3 residue in $LRR_{2,3}$ is negative in FSHr (E76) and LHCGr (E79) and positive in TSHr (K58) to interact with a complementary positive amino acid at the 'C-terminal loop' of FSH (R97)

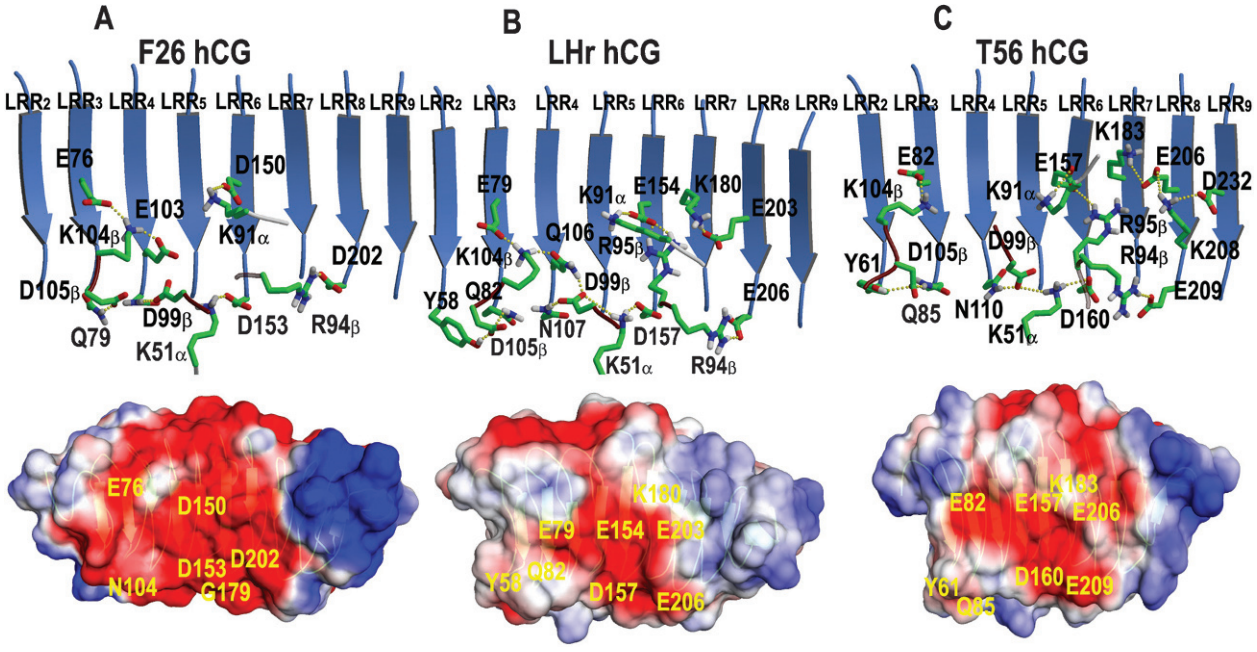

Figure 3. The interaction between hCG and (*A*) the K104N/K179G mutant FSHr, (*B*) wild-type LHCGr, and (*C*) K58S/E61Y/R80K/Y82E/I85Q/Y206E/N208K/K209E mutant TSHr; and the molecular electrostatic potential at the concave inner surface of the receptor.

and CG (K104) and negative in TSH (E118). On the other side, the X_5 amino acid in $LRR_{7,8}$ is positive in FSHr (K179) and TSHr (K209) and negative in LHCGr (E206) to appropriately interact with a negative amino acid at the 'determinant loop' of FSH (D90) and TSH (D111) and positive in hCG (R94). Thus, the 'C-terminal loop' and 'determinant loop' domains of GpHs contain different positive-negative (R97-D90 in FSH), positive-positive (K104-R94 in hCG), or negative-negative (E118-D111 in TSH) electrostatic patterns to interact in a selective manner with amino acids in the GpHRs with matching electrostatic properties: negative-positive (E76-K179 in FSHr), negative-negative (E79-E206 in LHCGr), or positive-positive (K58-K209 in TSHr), respectively. The interaction between the 'C-terminal loop' and the bottom X_5 part of LRR_2 is also divergent in the GpH-GpHR couples. The FSH-FSHr contains a hydrophobic-hydrophobic interaction (L99-L55), the hCG-LHCGr forms a polar hydrogen bond interaction (Y58/Q82-D105), and the TSH-TSHr forms an ionic interaction (E61-K121). These are the most important elements for the specificity of binding of glycoprotein hormones to their receptors.

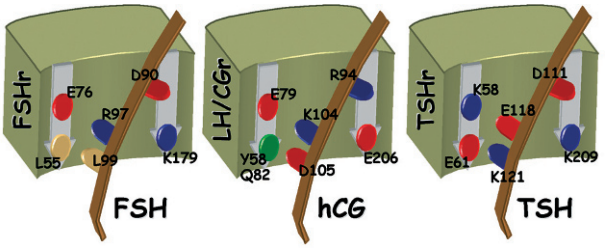

Figure 4. Schematic representation of the key important discriminating determinants for selective binding in the FSHr-FSH (left), LHCGr-CG (middle), and TSHr-TSH (right) couples. The 'seat-belt' segments of GpHs and the LRRs of GpHRs are shown in brown and gray, respectively. Negatively charged amino acids are colored in red, positively charged in blue, polar in green, and hydrophobic in light brown.

Natural mutations in the ECD of TSHr and FSHr affecting hCG specificity

Point mutations resulting in the K183R substitution in the TSHr (X_3 of LRR_7) or S128Y in the FSHr (X_3 of LRR_7), both located at the ECD domain (Fig. 1), were found in patients presenting gestational hyperthyroidism [28] or ovarian hyperstimulation syndrome [32], respectively. These natural mutants showed a clear lowering of their specificity towards hCG with an increase of sensitivity and affinity towards this hormone [32, 35]. Mutation of K183 to R (or to any other amino acid) releases E157 from the neutralizing interaction with K183 [35], permitting the interaction of R95 of hCG with the acidic E157 of TSHr, whereas mutation of S128 to Y permits FSHr to hydrogen bond R95 of hCG [32]. Notably, these two natural mutations in the ECD of the TSHr or FSHr, increasing their sensitivity towards hCG, cause their illegitimate gain of function by interacting with the same, R95, residue of hCG.

The role of receptor constitutive activity in hormone specificity

GpHRs [25] as well as others GPCRs [45] are known to adopt various conformations in response to ligands, breaching from fully inactive conformations, stabilized by inverse agonists, to active conformations, stabilized by agonists. The active conformation of GPCRs may also be induced by mutations of the receptor leading to constitutive, agonist-independent, signaling of GPCRs [46, 47]. Many naturally occurring constitutively active mutations in the heptahelical domain or the hinge region of GpHRs are known [48–50]. In human the wild-type TSHr displays significant constitutive activity and it is easily activated by mutations; the LHCGr is virtually silent but it is susceptible to activation by mutations, whereas the human FSHr receptor is totally silent in the absence of stimulation by agonists and more resistant to activation by mutations. Recently, several different FSHr gene mutants displaying measurable basal activity (T449I/A, I545T, and D567G/N, see Fig. 1) were found in patients affected with familial spontaneous ovarian hyperstimulation syndrome [29–31]. Importantly, these constitutively active mutants were shown to display increased responsiveness to both CG and TSH as compared with the wild-type FSHr [34], despite being located in the central and intracellular regions of the transmembrane helices, *i.e.*, clearly outside the ECD domain responsible for the high affinity and selective GpH binding (see Fig. 1). Thus, there is a relation between constitutive activity and lowering of specificity in the FSHr mutants, suggesting that loss of specificity of these mutants to hCG could be due to lowering of an intramolecular barrier to activation rather than to increase in binding affinity [4]. This view is fully compatible with the concept that the actual agonist of the heptahelical segment of the receptor may be the "activated" ectodomain [4]. It implies that the wild-type ectodomain of the FSHr displays some affinity for hCG, which, under physiological conditions, would be too low to cause promiscuous stimulation, but may become effective in the presence of a mutated (pre-activated) heptahelical domain, or abnormally high concentrations of hCG (*e.g.*, following hormone administration for induction of ovulation).

The role of tyrosine sulfation in hormone specificity

We have recently shown that, in addition to the specific interactions with the LRRs of the ECD domain, hormone binding requires ionic interactions involving sulfated tyrosine residues located in the so-called hinge region of the extracellular domain [51]. In the TSHr and LHCGr, *O*-sulfation of the first tyrosine residue of the highly conserved Y-D/E-Y motif located 27 residues upstream from transmembrane helix 1, is required to observe high-affinity interaction of these receptor with their cognate hormones. In contrast, the FSHr replaces this Y-D/E-Y motif by FDY, with sulfation of the second tyrosine being the functionally important event. The construction of chimeras between the FSHr and the LHCGr and extensive mutagenesis experiments provide strong evidence that structural differences in the hinge regions of these receptors also play a significant role in hormone-receptor-specific recognition [52]. The physiological relevance of tyrosine sulfation of the TSH receptor has recently been demonstrated in mice with loss of function mutation in Tpst2 gene, encoding one of the isoforms of tyrosylprotein sulfotransferase. The mutant mice develop dwarfism associated with profound hypothyroidism [53].

Perspective

Whereas in other GPCRs the binding of the agonists, within the N-terminal domain and the extracellular loops or/and the extracellular part of the transmembrane segments, is intimately linked with the activation of the receptor, in GpHRs, these two steps are separated. The publication of the crystal structure of the FSHr-FSH complex has opened the way to understanding the first of these steps: the specificity of binding of GpHs to their receptors at the molecular level, reviewed here. The increasing availability of animal genome and GpHR-GpH sequences will permit the evolution of ligand binding specificity to be further deciphered, in particular during the expansion of the chorionic gonadotropin gene family in primates. The process of GpHR activation and the subsequent G protein binding seem to be conserved among the other members of the rhodopsin-like GPCR family, considering the large number of conserved sequence patterns in the central and intracellular regions of the transmembrane segments [48, 54, 55]. Importantly, the intramolecular transduction of the signal between the hormone-bound ectodomain and the heptahelical segment of the GpHR family remains unknown [4]. The challenge is now to acquire direct structural data for this signal transduction mechanism, which is likely specific for the GpHR family.

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