Focus on Gonadotrophin Signalling

Specificity and promiscuity of gonadotropin receptors

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Abstract

The dichotomy between hormone recognition by the ectodomain and activation of the G protein by the rhodopsin-like serpentine portion is a well established property of glycoprotein hormone receptors. The specificity barrier avoiding promiscuous activation of the FSH receptor by the high concentration of human chorionic gonadotropin (hCG) prevailing during human pregnancy was thus believed to lie in the ectodomain. In the past two years, mutations responsible for rare spontaneous cases of ovarian hyperstimulation syndromes have partially modified this simple view. Five naturally occurring mutations have been identified which cause an increase in the sensitivity of the FSH receptor to hCG. Surprisingly, these mutations are all located in the serpentine portion of the receptor. In addition to their effect on sensitivity to hCG, they increase sensitivity of the FSH receptor to TSH, and are responsible for activating the receptor constitutively. Together, the available information indicates that the ectodomain and the serpentine domain of the FSH receptor each contribute to the specificity barrier preventing its spurious activation by hCG. While the former is responsible for establishment of binding specificity, the latter introduces a novel notion of functional specificity.

Recent data demonstrate that LH and FSH receptors can constitute functional homo- and heterodimers. This suggests the possibility that in cells co-expressing the two receptors, such as granulosa cells, the heterodimers might be endowed with functional characteristics different from those of each homodimer.

Introduction

Amongst the very large family of rhodopsin-like G protein-coupled receptors (GPCRs), glycoprotein hormone receptors (GpHRs) constitute a three-member subgroup made up of the follitropin receptor (FSHR) (Dias et al. 2002), the lutropin receptor (LH/CGR) (Ascoli et al. 2002) and the thyrotropin receptor (TSHR) (Szkudlinski et al. 2002). These are themselves part of a subfamily of receptors characterized by an ectodomain containing leucine-rich repeat motifs (LRRs), in addition to the canonical heptahelical serpentine domain typical of GPCRs. They are called leucine repeat-containing receptors (LGRs) (Hsu et al. 2000) and contain the recently identified relaxin (LGR7) (Hsu et al. 2002) and insulin-like 3 receptors (LGR8) (Kumagai et al. 2002) together with the receptor to the insect melanizing hormone bursicon (DLGR2) (Luo et al. 2005, Mendive et al. 2005) and orphan receptors.

The bipartite structure of GpHRs and LGRs is accompanied by a functional dichotomy: their LRR-containing ectodomain is responsible for the specificity of binding of their respective agonists, which translates into activation of the rhodopsin-like serpentine domain, itself responsible for transducing the signal within the cell, mainly via activation of the G protein Gs. According to a model elaborated initially for the TSHR (Vlaeminck et al. 2002), and later extended to the GpHR family (Vassart et al. 2004, Karges et al. 2005), binding of the hormone to the receptor would trigger a conformational change in a motif of the ectodomain, transforming it into an agonist of the serpentine domain. This model does not require the postulation of a direct interaction of the agonist with the extracellular loops or transmembrane helices of the serpentine domain in order to trigger activation, which has implications for the understanding of the mechanisms of inappropriate stimulation of the FSHR by chorionic...
gonadotropin in spontaneous ovarian hyperstimulation syndrome (OHSS) (see below).

Co-evolution of glycoprotein hormones and their receptors

Glycoprotein hormones are dimers with a common alpha subunit and hormone-specific beta subunits encoded by paralogous genes (i.e. descending from a common ancestor). This explains why the beta subunits of thyrotropin (TSH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) share about 40% sequence identity (see Fig. 1). The corresponding receptors are also encoded by paralogous genes and, accordingly, they also display about 40% sequence identity in their hormone-binding ectodomain (Fig. 1). This fits nicely with the notion that the hormone–ectodomain couples experienced co-evolution, resulting in tight binding specificity and avoiding promiscuous cross-signaling between the three endocrine systems (Moyle et al. 1994). In contrast, the serpentine ‘effector’ domains of the three receptors share higher sequence identity (about 70%), which suggests that they fulfill essentially the same function – activation of Gs. Whereas this conclusion is supported by domain swapping experiments (Braun et al. 1991), it must be kept in mind that additional signaling functions specific to each receptor, and distinct from the mere activation of Gs, are likely encoded in the serpentine domains (e.g. see Nechamen et al. 2004).

Chorionic gonadotropin in primates challenges the specificity of the system

The specificity barriers between the TSH–TSHR, FSH–FSHR and LH–LHR hormone receptor couples is such that no cross-signaling occurs under physiological conditions in which hormone concentrations are low (i.e. in the picomolar range). In diseases, however, the situation may be different. For instance, the extremely high TSH values observed in congenital hypothyroidism have been implicated in cases of precocious puberty as the consequence of promiscuous activation of the FSHR by TSH (Anasti et al. 1995). It is not widely appreciated that chorionic gonadotropin (CG), which achieves high concentrations during human pregnancy, is a relatively recent ‘invention’ of evolution. Except for the equidae, which have also evolved a chorionic gonadotropin (Murphy & Martinuk 1991), CG appeared in primates in which its concentration (and, likely, its role) increased to reach high nanomolar circulating concentrations during the first trimester of human pregnancy. The beta subunit of human CG (hCG) is 80% identical to beta LH (hCG and LH stimulate the same LH/CG receptor), and the disparity between the serum concentrations of TSH, FSH and LH on the one hand, and CG on the other, constitutes a

![Figure 1](image)  The beta subunits of the glycoprotein hormones and the glycoprotein hormone receptors are encoded by paralogous genes. Sequence identities are indicated separately for the ectodomains and the serpentine domains of the three receptors (left) and for the beta subunits of the four hormones (right). The pattern of shared similarities suggests co-evolution of the hormones and the ectodomain of their receptors, resulting in the generation of specificity barriers. The high similarity displayed by the serpentine portions of the receptors is compatible with a conserved mechanism of intramolecular signal transduction. Adapted from Vassart et al. (2004).
real challenge to the specificity barriers during the first trimester of human pregnancy. This is readily illustrated by the inverse relation between hCG and TSH concentrations observed during this period of normal pregnancy, demonstrating that hCG reaches concentrations at which it displays thyrotropic activity (Gliniopr 1997). Positively selected mutations have been identified in the alpha subunits of glycoprotein hormones of primates, resulting in lower potency of the complete hormones when compared with other mammals (Grossmann et al. 1997). This may be interpreted as a sign of balanced evolution between the negative effects which would result from cross-signaling (hyperthyroidism, ovarian hyperstimulation syndrome) and the still hypothetical positive effects of the high concentrations of hCG. These may be independent of the LH/CG receptor, since the concentrations achieved are well beyond saturation of its classical concentration action curve (but see below).

The binding specificity of GpHRs is clearly encoded in their amino terminal ectodomain

From early experiments in which the ectodomains of the LH and FSH receptors were exchanged (Braun et al. 1991), it is widely accepted that the LRRs of the ectodomain are the structures implicated in binding specificity. In addition, it has convincingly been demonstrated that purified ectodomains, truncated from their serpentine domains, do bind their cognate hormones with high affinity (Cornelis et al. 2001, Remy et al. 2001, Schmidt et al. 2001). Very recently, crystallization of a complex between FSH and the ectodomain of the FSHR has provided the first direct structural evidence for the residues implicated in recognition specificity at the atomic level of resolution (Fan & Hendrickson 2005). Extensive site-directed mutagenesis experiments, piloted by molecular modeling of the ectodomains, had previously identified key residues involved in the specificity of recognition of the TSH and FSH receptors. The observation that the specificity barriers could be virtually abolished by exchanging a very limited number of amino acids between the ectodomain of the TSHR (8 residues) or the FSHR (2 residues), and the LH/CGR demonstrate unambiguously that specificity is encoded in the portion of the ectodomains containing leucine-rich repeats (Smits et al. 2003a) (Fig. 2).

A nice illustration of this conclusion is provided by a natural mutation of the TSHR rendering it abnormally sensitive to hCG (Rodien et al. 1998). The mutation was identified in two women who experienced severe hyperthyroidism on the occasion of each of their pregnancies. It affects a residue, lysine in position 183, predicted to lie in the sixth LRR, on the hormone-binding surface of the TSHR. A convincing molecular explanation for the phenotype was reached by a combination of molecular modeling and site-directed mutagenesis experiments (Smits et al. 2002).

As a logical extension of these notions, mutations which would make the FSHR abnormally sensitive to hCG were searched for in spontaneous ovarian hyperstimulation syndromes. It was known that exceptionally high circulating levels of hCG, as observed for example in molar pregnancies, are capable of causing ovarian hyperstimulation via promiscuous activation of the FSHR (Ludwig et al. 1998). When FSHR genes were sequenced from patients displaying recurrent spontaneous OHSS, mutations were indeed found (Smits et al. 2003b, Vasseur et al. 2003, Montanelli et al. 2004a, 2004b) but, surprisingly, in all cases studied to date, the mutations affected residues in the serpentine domain (Fig. 3).

Functional specificity of the FSHR is encoded, in part, in the serpentine domain

The unexpected observation that mutations of the FSHR associated with spontaneous OHSS were located in the serpentine domain of the receptor prompted a detailed analysis of the phenotype of the mutants, when expressed by transfection of complementary DNA in cell lines. After an initial period of disagreement on some aspects of the phenotype (points 2 and 4 below; Smits et al. 2003b, Vasseur et al. 2003), we can summarize the characteristics of these natural mutant receptors in the following way: (1) they display increased sensitivity to hCG; (2) they also display increased sensitivity to TSH; (3) they keep normal sensitivity to FSH; and (4) in contrast to the wild-type FSHR, which is totally silent, they display detectable constitutive activity (Smits et al. 2003b, Montanelli et al. 2004a, 2004b). The location of the mutations in the topology of the receptor (Fig. 3), together with the observation that the loss of specificity affected the response to both hCG and TSH, led to the hypothesis that the underlying mechanism might be related to an increased ability of the mutants to be activated, rather than to an increase in binding affinity. This hypothesis was directly supported by experimental evidence: artificial mutations with an effect on the basal activity of the FSHR, whether they affect the residues implicated in spontaneous OHSS or not, caused a similar loss of specificity for hCG and TSH (Montanelli et al. 2004b). In addition, there was a direct relation between basal activity of the mutants and the extent of their sensitivity to the promiscuous hormones (De Leener A, Montanelli L, Van Durme J, Chae H, Smits G, Vassert G and Costagliola S, unpublished observations). From these data, it has been proposed that the specificity barrier against activation of the FSHR by hCG evolved by locking the serpentine portion of the receptor in a completely silent (inactive) state (Vassart et al. 2004). As such, the wild-type receptor would be unable to respond to low affinity interaction of hCG (or TSH) with its ectodomain. In OHSS mutants, the intramolecular barrier to activation would be lower, thus allowing even poor agonists (such as hCG or TSH) to become effective (Fig.
In favor of such an interpretation, one of the residues affected by OHSS mutation (D6.30) (see Fig. 3) has been identified as a key player in the maintenance of rhodopsin-like GPCRs in the inactive state (Ballesteros et al. 2001). Mutations of the homolog residues in the TSHR or LH/CGR cause constitutive activation responsible for hyperfunctioning thyroid adenomas and autosomal dominant hyperthyroidism (Parma et al. 1993, Duprez et al. 1994), or male-limited pseudoprecocious puberty (Shenkjer et al. 1993, Themmen & Huhtaniemi 2000) respectively.

If the above hypothesis is correct, one might expect non-primate FSHRs to display some sensitivity to hCG, when tested ex vivo, together with some basal activity. This is indeed the case, as suggested by the phenotype of chimeric receptors containing the ectodomain of the human FSHR pasted upstream of a serpentine domain from a series of non-primates species (De Leener et al., unpublished observations). Most chimeras displayed increased sensitivity to hCG when compared with the wild-type human FSHR. Interestingly, the relation between basal activity and relaxed specificity seems to be a characteristic of the FSHR. Mutants of the TSHR endowed with increased constitutive activity do not show a significant increase in sensitivity to hCG (Montanelli et al. 2004b). It must be noted, however, that contrary to the FSHR, the wild-type human TSHR exhibits easily measurable constitutive activity (Parma et al. 1993). It seems, therefore, that

Figure 2 Illustration of the specificity barrier encoded in the ectodomain of the FSH receptor. Substitution of two residues of the ectodomain of the FSHR (K104N, K179G) increases dramatically its response to hCG. The left panel shows responsiveness to hCG in terms of cAMP production of the wild-type FSHR (black circles), the K104N (green diamonds), the K179G (blue circles) and the double K179G/K104N mutant (red circles) after transfection in COS-7 cells. The responsiveness of the wild-type LH/CGR receptor is shown for comparison (black squares). The right panel illustrates the position of K104 and K179 on the tridimensional structure of the ectodomain of the FSHR (Fan & Hendrickson 2005).

Figure 3 Schematic representation of the FSH receptor, with indication of the mutations identified in patients with spontaneous ovarian hyperstimulation syndrome: D6.30N (Smits et al. 2003b); D6.30G (De Leener et al., unpublished observations; see page 3); T3.32I (Vasseur et al. 2003); T3.32A (Montanelli et al. 2004a); I5.54T (De Leener et al., unpublished observations). The standardized numbering system used to identify amino acids is from Ballesteros & Weinstein (2002).
evolution has followed distinct paths in the TSHR and FSHR to cope with the emergence of chorionic gonadotropin. In the former, maintenance of specificity relied on evolution of the binding barrier within the ectodomain. In the latter, an intramolecular barrier to activation was increased by strengthening silencing locks of the serpentine domain.

Additional complexity from dimerization of receptors

Over the past few years, the notion that GPCRs are present in the plasma membrane as dimers or oligomers has progressively become the prevalent view (Fotiadis et al. 2003, Terrillon & Bouvier 2004). In addition, the possibility that receptors belonging to the same or close subfamilies can associate into heterodimers has been convincingly documented. The GpHRs are no exception (Osuga et al. 1997, Horvat et al. 2001, Ji et al. 2002, Latié et al. 2002, Tao et al. 2004, Urizar et al. 2005). There were few arguments, however, for a possible functional significance of homo- or hetero-dimerization of GpHRs until recently, when it was shown that dimerization was associated with allostery, more precisely with negative cooperativity (Urizar et al. 2005). Briefly stated, these novel data contend that GpHRs are present in the plasma membrane as homodimers and that binding of the hormone to one subunit of the dimer decreases strongly the affinity of the other for the ligand. When two different GpHRs are present in the same cell, transfection experiments showed that they are capable of forming heterodimers, and these also display negative cooperativity. This means that in cells co-expressing FSHR and LH/CGR, as granulosa cells do before luteinization (Hillier 2001), the possibility exists of an interplay between the two hormones at the level of putative receptor heterodimers. One consequence of these observations is that under most physiological conditions (i.e. at low agonist concentration) a single agonist would be bound per dimer. However, given the high concentration of hCG achieved during pregnancy, we must consider the possibility that dimers (be they homo- or hetero-dimers) could show different abilities to activate downstream regulatory cascades when they are engaged by a single or by two molecules of agonist.

Perspectives

It is hoped that the recent structural and functional data collected on gonadotropins and their receptors will open new perspectives for understanding the pathophysiology of a series of diseases, in addition to providing new
insights into the basic mechanisms involved in their action. In particular, we believe that the following points warrant further investigation: (1) the putative role of FSHR variants in the severity of iatrogenic OHSS (Daelemans et al. 2004); (2) the role of putative FSHR/LHR heterodimers in granulosa cells at the time of ovulation; and (3) the putative role of LHR dimers as the target for the high, saturating concentrations of hCG during pregnancy.

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