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The thyrotropin receptor, a GPCR with a built-in inverse agonist

Gilbert Vassart*, Sabine Costagliola

Faculty of Medicine, Institut de Recherche Interdisciplinaire (IRIBHM), Free University of Brussels, Campus Erasme, 808 route de Lennik, B-1070 Brussels, Belgium

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Abstract

The thyrotropin receptor (TSHr) is a member of the glycoprotein hormone receptors (GPHR), themselves, part of the rhodopsin-like G protein-coupled receptor family. The GPHR are characterized by a large aminoterminal extracellular extension responsible for the recognition and binding of their dimeric 30 kDa agonists (glycoprotein hormones thyrotropin (TSH), lutropin/chorionic gonadotropin (LH/CG) or follitropin (FSH)). This ectodomain is composed of a central portion made of nine leucine-rich motifs and two flanking domains containing several disulfide bridges. In addition of encoding the specificity for hormone recognition, the ectodomain has been shown to exert an inhibitory constraint on the constitutionally active serpentine portion of the receptor coupled in priority to Gsα. This conclusion was reached from experiments with receptor constructs harboring truncated ectodomains, which displayed an increase in their constitutive activity. When compared with the wild type receptor maximally stimulated by TSH or the most active mutants of the ectodomain (e.g. Ser281Leu), the truncated constructs showed only partial activation. Interestingly, the "beheaded" receptor could be further activated by mutations affecting the transmembrane helices of the serpentine but not by selected mutations in the exoloops of the serpentine, which are known to be potent activators of the holoreceptor. From these observations, we propose a model for TSHr activation in which binding of the agonist would switch the ectodomain from a tethered inverse agonist into a tethered agonist.

Keywords: GPCR; Glycoprotein hormone receptors; Constitutive activation; Inverse agonist; 7TM receptors

* Tel.: +32-2-555-4145; fax: +32-2-555-4212.
E-mail address: gvassart@ulb.ac.be (G. Vassart).

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1. Introduction

For small molecules like the biogenic amines, there is strong evidence that the agonists of GPCRs interact directly with specific residues of the transmembrane helices of the receptor. For neuropeptides and small protein agonists like neurokinins, it is believed that interaction involves both exoloops and aminoterminal portion of the receptors, in association with residues of the transmembrane helices. The situation is less clear for receptors to the glycoprotein hormones thyrotropin (TSH), lutropin/chorionic gonadotropin (LH/CG) and follitropin (FSH). In these cases, the agonists are bulky dimers of about 30 kDa made of a common alpha subunit and hormone-specific beta subunits [1,2]. The corresponding receptors contain a canonical serpentine portion, with seven transmembrane helices typical of rhodopsin-like GPCRs, and a large (350–400 residues) aminoterminal ectodomain containing leucine-rich repeats [3–6]. The aminoterminal segments are responsible for high affinity binding of the hormones and recognition specificity. How binding of the hormone to the ectodomain results in activation of the serpentine portion of the receptor is still unknown. It has been proposed that following high affinity binding of the hormone to the ectodomain, low affinity interaction between the hormone and the exoloops or, even, residues of the transmembrane helices would result in activation.

2. Structure–function relations of the ectodomain

The ectodomain contains a central portion of nine leucine-rich repeats (LRR) bordered by two cysteine-rich domains, typical of the large family of LRR proteins [7]. Structural models of the extracellular part of the glycoprotein hormone receptors based on the atomic structure of the porcine ribonuclease inhibitor [8] have been proposed [9,10]. According to these models, each LRR comprises 20–24 amino acids forming a β strand followed by an α helix. The LRR units are arranged with their β strands and α helices parallel to a common axis and organized spatially to form a horseshoe-shaped structure with the β strands and α helices making the concave and convex surfaces of the horseshoe, respectively. By analogy with the atomic structure of the ribonuclease–ribonuclease inhibitor complex [8], it is assumed that hormones make contact, mainly but not exclusively, with the β sheets of the inner concave portion of the horseshoe. Experiments in which individual residues of the horseshoe are exchanged between the glycoprotein hormone receptors strongly support this model. Exchanging, respectively, two or eight residues of the ectodomains of the TSHr or FSHr for the corresponding residues of the LH/CGr results in mutant constructs displaying sensitivity to hCG identical to that of the wt LH/CGr (G. Smits et al., in preparation).

In addition to specific interactions of the hormones and the horseshoe portion of the receptors, it has recently been shown that tyrosine sulphation at specific sites of the ectodomain of the receptors is required for high affinity binding [11].

These kinds of experiments cast light on the binding characteristics of the glycohormone receptor family but give no information on the mechanisms involved in the subsequent (or concomitant) activation of the serpentine. In the ribonuclease inhibitor, binding of ribonuclease causes a minimal change in the curvature of the horseshoe. Whether something similar happens in the glycoprotein hormone receptors is unknown.

3. The serpentine domain of the TSH receptor (TSHr) is activated by a panel of gain of function mutations

In contrast to the LH/CGr and FSHr, the TSHr has been shown to present significant basal activity in the absence of ligand [12,13]. In addition, more than 25 naturally occurring mutations have been found to increase dramatically TSHr constitutive activity, leading to acquired diseases such as toxic adenomas or (rarely) thyroid cancer, and autosomal dominant forms of hereditary hyperthyroidism [14]. Quite unexpectedly, these mutations have been located in all places of the receptor: the ectodomain (see below), the extracellular and intracellular loops, or the TM region. Interestingly, such activating mutations are less frequently found in the LH/CGr and the spectrum of residues involved seems to be narrower [15]. For the FSHr, no convincing natural mutant has been identified and the receptor is resistant to activation by many amino acid substitutions with a strong activating effect, when present in homologous segments of the TSH or LH/CG receptors [16].

The majority of these mutations affect residues of the transmembrane helices and cytoplasmic loops. The mechanisms by which they lead to increase in constitutive activity have remained largely unknown. However, recent experiments exploiting molecular models of TSHr serpentine domain, elaborated on the basis of the 3D structure of rhodopsin, are yielding hypotheses which are currently being tested [17,18]. Despite sequence signatures that are specific to the glycoprotein hormone receptors, there is hope that understanding the activation mechanisms in the TSHr will provide information on the active conformation of the whole rhodopsin-like GPCR family.

4. Intramolecular signal transduction between the ectodomain and the serpentine domain of glycoprotein hormone receptors

The observation that mutations of specific residues of the ectodomain (serine 281) and the exoloops (isoleucine 486, isoleucine 568) constitutively activate the TSH receptor led to the hypothesis that the wild type ectodomain would exert an inhibitory constraint on the inherently "noisy" serpentine portion of the TSHr through interaction with the exoloops [19,20]. This hypothesis is in agreement with the observation that mild treatment of cells expressing the TSHr with trypsin results in ligand-independent activation, while simultaneously removing an epitope of TSHr ectodomain [21]. More recently, receptor mutants with truncations of their ectodomain have provided direct evidence supporting this model [22]. These experiments demonstrated that removal of the hormone-binding ectodomain causes an increase in basal activity as compared to the holoreceptor. They extend other results obtained with ectodomain-truncated TSHr mutants [23] and provide evidence that the aminoterminal portion of TSHr behaves as a tethered inverse agonist (Fig. 1). Whether this effect involves specific interactions, as suggested by the differential effects of the individual mutants, or is secondary to a mere stabilization of the structure of the serpentine, is presently unknown [24,25]. In this context, it is noteworthy that activating mutations have been shown to cause destabilization of GPCRs.

According to one model for activation of the LH/CGr, the ectodomain would bind the hormone with high affinity and "present" it to the serpentine for a low affinity productive

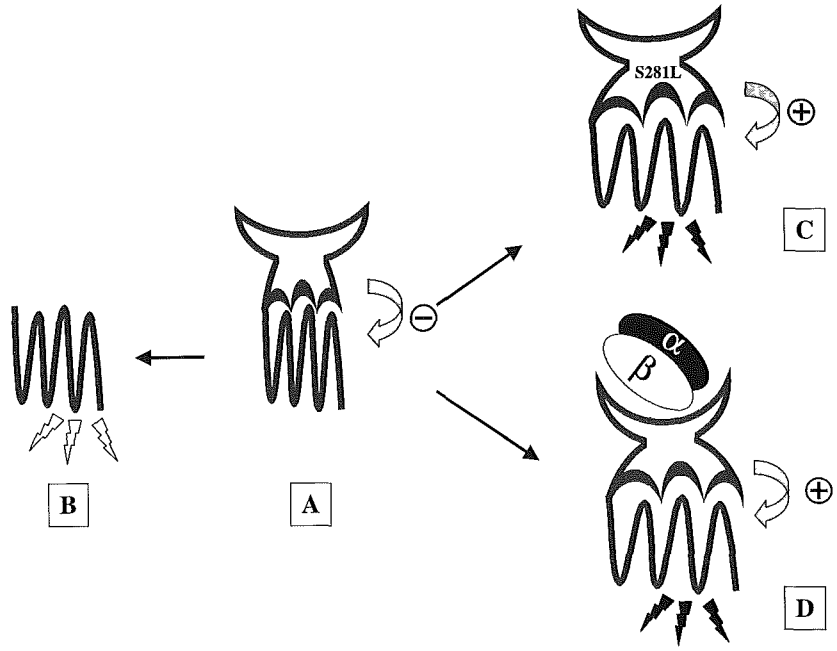

Fig. 1. Putative model of the intramolecular interactions involved in the activation of the TSH receptor. (A) The basal state of the receptor is characterized by an inhibitory interaction between the ectodomain and the serpentine domain. The ectodomain would function as a tethered inverse agonist. (B) Removal of the ectodomain releases the serpentine domain from the inhibitory interaction, resulting in partial activation. (C) Mutation of Ser281 into Leu switches the ectodomain from an inverse agonist into a full agonist of the serpentine domain. (D) Binding of TSH to the ectodomain is proposed to have a similar effect, converting it into a full agonist of the serpentine portion. It must be stressed that the scheme is purely illustrative. It emphasizes that, according to our model, activation does not require a direct interaction between the hormone and the serpentine domain. Such an interaction, however, is by no means excluded by our experiments (reproduced from Ref. [22], with permission).

interaction resulting in activation [26,28]. None of our truncated TSHr mutants could be stimulated even by a saturating concentration of TSH which does not support the above model.

In elegant experiments involving chimeras between the LH/CGr and LGR2, a relatively distant member of the glycohormone receptor family of drosophila, the group of Hsueh provided evidence that the silencing interaction between the ectodomain and the serpentine involved interactions between the "hinge region" of the ectodomain (an imperfectly conserved segment downstream from the last leucine-rich repeat) and the second exoloop of the serpentine [27].

From these observations, it was initially proposed that activation of the TSHr (and, by analogy of the other glycoprotein hormone receptors) would result from the mere release of the serpentine domain from inhibitory interaction(s) exerted by the ectodomain [28]. Experiments comparing the increase of constitutive activity observed in N-terminally truncated constructs (ca. 5-fold) with the activity reached by some point mutants with substitutions in the hinge region of the ectodomain (over 20-fold), demonstrated that this

simple model was inapplicable. They indicated that when mutated in Ser281, the ectodomain switches from an inhibitor of the activity of the serpentine (a tethered inverse agonist) into an activator [a full(?) agonist]. Reminiscent of the situation prevailing for activation of the serpentine, there is indication that the conformational change of the ectodomain associated with activation corresponds to a "gain of function" resulting from a "loss of structure", locally. This is suggested by experiments showing a direct relation between the rate of activation and the extent of disorganization of local structure predicted to occur in a panel of Ser277 mutants (the homologue of Ser281 in the LH/CGr) [29,30]. According to our current model, activation of the serpentine domain of the glycoprotein hormone involves a composite module encompassing a portion of the ectodomain (the extent of which is still undefined) and the exoloops of the serpentine. This conclusion was reached from the observation that activating mutations involving residues of the exoloops were ineffective when introduced in a mutant receptor devoid of ectodomain [22]. On the contrary, activating mutations affecting residues of the transmembrane helices were equally effective whether introduced in serpentine-alone constructs or the holoreceptor [22].

Although still conjectural, it seems likely that activation of the receptor by its normal agonist (or by autoantibodies, in patients with Graves' disease) would similarly trigger metamorphosis of the ectodomain from an inverse agonist into an agonist of the serpentine. This hypothesis makes sense in an evolutionary perspective and provides an explanation to the observation that chimeras made of the ectodomain of the human LH/CGr and the serpentine of LGR2 (a very distant homologue in drosophila) can be activated by hCG [27]. The structural changes induced by binding of the hormone to the leucine-rich repeats and the subsequent triggering of the switch remain to be identified.

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