

Inverse relation between constitutive activity and negative cooperativity in mutants of glycoprotein hormone receptors.

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The glycoprotein hormone receptors (GPHRs) constitute a subfamily of rhodopsin-like G protein-coupled receptors (GPCRs) responsible for signal transduction by thyrotropin (TSH), lutropin or chorionic gonadotropin (LH/CG), and follitropin (FSH). They are primarily G α_s coupled receptors, with a division of labor between agonist binding, by a large ectodomain made of leucine-rich repeats, and activation of the G protein, by a canonical heptahelical serpentine domain. A large number of natural activating mutations have been identified in GPHRs which are responsible for a variety of endocrine phenotypes. In addition extensive site-directed mutagenesis has been performed yielding a particularly wide panel of constitutively active GPHR mutants (CAMs). Direct structural information is only available for the leucine-rich repeat portion of the ectodomain of the FSHR and TSHR. For the serpentine portions of GPHRs, molecular modeling must rely on the crystal structures of rhodopsin and the β_2 adrenergic receptor.

Activation of GPHRs deviates from the mechanisms implicated in the activation of the majority of GPCRs devoid of large ectodomains. Whereas direct interaction between the agonist and portions of the serpentine domain is thought to take place for the majority of GPCRs (refs a chosir), a favored current model holds it that activation of GPHRs involves switching of the ectodomain into a tethered agonist of the serpentine portion, upon binding of the hormones to the ectodomain. This model provides a rational explanation to the observation that the TSHR can be activated by binding of ligands with little if any sequence identity (TSH, thyrostimulin, autoantibodies) as well as by point mutation in the ectodomain.

Homo- or hetero- dimerization/oligomerization have become central themes of GPCR research. Evidence for dimerization of GPHRs are convincing and, together with data from chemokine receptors, provided amongst the first experimental illustration of the allosteric correlate of GPCR dimerization. Strong negative cooperativity was demonstrated in both kind of receptors, leading to the notion that a single protomer is activated under conditions of physiological agonist concentrations.

From a topological point of view, negative cooperativity implies introduction of asymmetry upon binding of the agonist to a dimer. This poses the question of the overall stoichiometry between agonist(s), receptor(s) and the G protein(s), and about the conformational changes achieved in individual protomers upon activation. In the present study we took advantage of the wide panel of activating mutations available for the TSH receptor to explore the effect of constitutive, i.e. symmetrical, activation of the mutants on their allosteric behavior. Our results demonstrate unambiguously an inverse relation between constitutive activity and negative cooperativity.