

Insights learned from L457R, an activating mutant of the hLHR

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L457R is a naturally occurring activating mutation of the hLHR identified in a young boy with gonadotropin-independent precocious puberty. This mutation results in the substitution of a highly conserved leucine in TM3 of the hLHR with an arginine. When expressed in 293 cells, L457R causes ~10-fold increase in basal cAMP levels and it exhibits complete unresponsiveness to further hormonal stimulation in spite of having normal hCG binding affinity. When membranes are isolated from cells expressing L457R they similarly exhibit an increase in basal adenylyl cyclase and a lack of further activity upon hCG stimulation. These data suggest that the lack of hormonal responsiveness is intrinsic to the structure of the mutant receptor and not to post-receptor events, such as increased receptor internalization, that may modify the responsiveness of cells expressing this mutant. The young boy, who was heterozygous for this mutation, exhibited elevated testosterone levels that were not increased further upon exogenous hCG treatment. This finding was surprising given the expectation that the wt hLHR encoded by the other allele would exhibit hormone responsiveness. Therefore, studies were performed examining the co-expression of the L457R activating mutant with the wt hLHR. It was determined that L457R causes an attenuation of hCG-stimulated cAMP by the wt hLHR and that this is due to the activation of a cAMP responsive phosphodiesterase. This stimulation of phosphodiesterase activity was observed with other activating mutants as well. This adaptive response to the increased basal levels of cAMP causes a heterologous desensitization of hormone-stimulated cAMP by the hLHR as well as other Gs-coupled receptors.

The L457R and other activating mutants of the hLHR display decreased protease sensitivity as compared to the unliganded wt receptor, supporting the hypothesis that the conformation of the constitutively active hLHR is distinct from the resting wt state. To gain further insights into the molecular basis for the constitutive activation and lack of hormonal responsiveness of the L457R mutant, other substitutions of L457 were examined. Only positively charged residues at this site conferred activation and reduced hormonal responsiveness. Molecular dynamic simulations of L457R as compared to wt hLHR (performed by Francesca Fanelli) suggested that the arginine introduced at codon 457 in TM3 forms a salt bridge with D578 in TM6 and, similar to other activating mutations of the hLHR, this results in an increased solvent accessible surface in the cytoplasmic regions of TMs 3 and 6. Disruptive and reciprocal mutagenesis studies confirm the formation of a salt bridge between R457 and D578 and that this salt bridge is responsible for both the constitutive activity and hormonal unresponsiveness of L457R. These results, which demonstrate the requirement for a salt bridge between TMs 3 and 6 to stabilize the active L457R, challenge the current paradigm of constitutive activation arising as a result of disruption of interhelical interactions that stabilize the resting state.