

Role of the intracellular domains of the human FSH receptor in G_s protein coupling and receptor cell surface membrane expression. Alfredo Ulloa-Aguirre¹, Aída Uribe¹, Teresa Zariñán¹, Ismael Bustos-Jaimes², Marco A Pérez-Solís¹ and James A. Dias³.

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The human FSHR belongs to the superfamily of G protein-coupled receptors (GPCRs). This receptor consists of 695 amino acid residues and is preferentially coupled to the G_s protein. This receptor is highly conserved among species (overall homology, 85%), with a 25 %-69 % homology drop when compared to the human LH and TSH receptors. Although studies in prototypical rhodopsin/ β -adrenergic receptors suggest that multiple domains in the intracellular loops (IL) and the carboxyl-terminus (Ctail) of these receptors contribute to G protein coupling and receptor expression, there is a paucity of structure/function data on the role of these domains in FSHR function. Employing point mutations we have found that several residues present in the IL-2 of the FSHR are important for both coupling the receptor to the G_s protein and maintaining the receptor molecule in an inactive conformation. In fact, HEK-293 cells expressing several FSHR mutants with substitutions at R450 (central to the highly conserved ERW triplet motif) and T453 (a potential target for phosphorylation) failed to mediate ligand-provoked G_s protein activation but not agonist binding, whereas substitutions at the hydrophobic L460 (a conserved residue present in all glycoprotein hormone receptors) conferred elevated basal cAMP to the transfected cells. Thus, this particular loop apparently acts as a conformational switch for allowing the receptor to adopt an active conformation upon agonist stimulation. Residues in both ends of the IL-3 are important for signal transduction in a number of GPCRs, including the FSHR. We have recently explored the importance of the reversed BBXXB motif (BXXBB; where B represents a basic residue and X a non-basic residue) present in the juxtamembrane region of the FSHR IL-3. A FSHR mutant with all basic amino acids replaced by alanine failed to bind agonist and activate effector, and was expressed as an immature \leq 62 kDa form of the receptor as disclosed by Western blots. Individual substitutions of basic residues resulted in mutants that bound agonist normally but failed to activate effector when replaced at R552 or R556. Triple mutations in the same motif located in the NH₂-end of the Ctail resulted in a complete inability of the receptor to bind agonist and activate effector, whereas individual substitutions resulted in decreased or virtually abolished agonist binding and cAMP accumulation, with both functions correlating with the detected levels of mature (80 kDa) forms of the receptor. All IL3 and IL4 mutants internalized following the pattern exhibited by the Wt FSHR. Thus, the BXXBB motif at the IL3 of the FSHR is essential for coupling the activated receptor to the G_s protein, whereas the same motif in the Ctail is apparently more important for membrane expression. Finally, the role of cysteine residues present in the Ctail has been analyzed. Alanine replacement of C629 and C655, but not C627, resulted in normal cell surface membrane expressed FSHR mutants with reduced (~15 %-30 %) efficiency to activate effector. C629 and C655 may be targets for palmitoylation since molecular modeling did not predict the formation of a disulfide bond between these two residues. (Supported by grant 38056-M from CONACyT, Mexico, and NIH HD18407).