Dominant Negative Effects of Human Follicle-stimulating Hormone Receptor (hFSHR) Mutants on Wild type Receptor Expression

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A large body of experimental evidence indicates that G protein-coupled receptors (GPCRs) form homo- and/or hetero-oligomeric complexes in cells. Homodimerization may affect biogenesis and membrane targeting of the complexed receptors. We have analyzed whether hFSHR mutants with complete or partial expression defects may exert dominant negative actions on the cell surface membrane expression of the wild-type (WT) hFSHR, as has been found for other GPCRs. In human embryonic kidney-293 cells cotransfected with constant amounts of WT receptor cDNA and increasing concentrations of each hFSHR cDNA mutant [R556A, R618A (complete expression defect), and K614A (partial expression defect)], we found that agonist-stimulated cAMP accumulation progressively decreased; maximal responses declined by 80% in cells expressing a relative excess (6 to 7 fold) of the R556A and R618A mutants, and by 40% in cells expressing a six-fold excess of the K614A mutant. Both binding of ^{125}I-FSH to the WT receptor and expression levels of the hFSHR 80 kDa form of the receptor (that represents the mature, fully glycosylated hFSHR) also showed a progressive decrease by cotransfecting mutant and WT hFSHR cDNAs. The cAMP response, ^{125}I-FSH binding and expression of the 80 kDa species of the receptor were restored when the K614A mutant was coexpressed at the highest concentration WT:mutant ratio (1:7), suggesting either intracellular association between the mutant hFSHR species or increased expression of the mutant receptor. These results were confirmed by immunofluorescence microscopy using a WT hFSHR-green fluorescent protein chimera cotransfected with the mutant receptors. The dominant negative effect of the hFSHR mutants was not observed when the WT receptor was cotransfected with a hFSHR/rat LHR COOH-terminus chimera, the human β_2-adrenergic- or the dopamine D_1 receptor (G_s-coupled), a gonadotropin-releasing hormone receptor (GnRHR)/Catfish GnRHR COOH-terminus quimera ($G_{q/11}$-coupled) or the human thyrotropin receptor (G_s-coupled). Mutant hFSHRs also provoked dominant negative actions when coexpressed with the WT luteinizing hormone receptor; this effect, however, was less pronounced than that observed on the WT hFSHR species. These results demonstrate that expression-defective hFSHRs exert dominant negative actions on WT receptor function, as found in other GPCRs. Defective intracellular transport or interference with proper maturation due to the formation of misfolded complexes between the receptor species may explain the observed effects provoked by the altered hFSHRs.
(Supported by grants 2006/1A/I/008 from the FOFOI-IMSS, grant 45991 from CONACyT, Mexico, and by NIH grants HD-19899, RR-00163 and HD-18185).