

Effects of modifying FSH receptor adapter protein interaction domains on FSH signaling.

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FSH the primary stimulus for growth and differentiation of ovarian follicles activates many receptor signaling pathways in granulosa cells. It is likely that signaling endosomes and macromolecular platforms provide opportunities to coordinate temporally and spatially restrict signaling. The cytoplasmic face of the FSH receptor includes four intracellular loop domains which can participate in formation of signaling complexes. Intracellular loops 1 and 2 are interaction sites for two adapter proteins, APPL1 and 14-3-3 tau respectively. Three amino acid substitutions abrogate APPL1 association with the human FSHR intracellular loop 1 when measured in a yeast two hybrid assay. The FSHR-K376A mutant was selected for further analysis because its trafficking appeared most similar to wild type hFSHR when expressed in HEK293 cells and it failed to co-immunoprecipitate APPL1. When expressed in the human granulosa cell line KGN, FSHR-K376A evidenced normal estrogen and progesterone production when compared to wild type receptor. In contrast intracellular calcium release was minimal when individual cells were analyzed. Thus APPL1 association with FSHR intracellular loop one, appears to facilitate FSH induced production of intracellular messengers, such as inositol 3-phosphate (IP₃). Single amino acid substitutions of the 14-3-3 tau interaction domain of FSHR resulted in loss of function or gain of function interaction measured using a yeast two hybrid assay. One of the interaction gain of function mutants hFSHR-H452A trafficked to the cell surface normally and bound FSH. Remarkably, this mutation resulted in a loss of function for cAMP production. In view of studies which showed that hFSHR-H452A was not internalized following hormone binding, these data suggest that 14-3-3 can modify receptor internalization and signaling potential upstream of the protein kinase A pathway, possibly by blocking the association of G-proteins with the FSHR. This mutant will allow for unprecedented analysis of cAMP and internalization independent FSH signaling. Site specific mutations of protein interaction domains which affect FSHR function point to subtleties of the FSH response, which may occur in vivo. Targeting these specific interactions may lead to selective FSH response modifiers.

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