

Selective β -arrestin-dependent signalling by FSHR mutants.

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Mutations in FSH or its receptor can alter or suppress fertility in both sexes. FSH binds to and activates a G protein-coupled receptor expressed by Sertoli or granulosa cells. Classically, the FSHR couples to Gs, induces cAMP production, PKA activation and subsequently a wide range of downstream signalling pathways including MAP Kinase ERK. Our laboratory has recently shown that both β arrestins and cAMP/PKA are able to activate MAP Kinase ERK upon FSH stimulation, with differences in kinetics (Kara E et al., Mol Endocrinol, 20:3014, 2006). This led to the hypothesis that both pathways are independent and trigger distinct biological outcomes. In this context, our goal was to develop FSHR mutants selective of β -arrestin-dependent pathway for ERK activation in order to determine i) the contribution of this pathway in FSH-induced cellular responses and ii) its significance relative to the classical cAMP/PKA mechanism in reproductive physiology.

Materials and methods: By using evolutionary tracing (Madabushi S et al. J Biol Chem 279:8126, 2004), R466 and T469 residues located in the second intracellular loop have been predicted to be important for Gs coupling to the FSHR upon stimulation. *In vitro* studies performed on rat FSHR mutants R466A and T469F confirmed that Gs-coupling was impaired (Timossi C. et al., Mol Cell Endocrinol, 189:157, 2002). We have introduced the R466A and T469F mutations in the mouse FSHR in order to evaluate their ability to selectively activate the β -arrestin pathway.

Results and discussion: Both mutants were compared to the wild type mouse FSHR permanently expressed in HEK 293 cell lines. The comparison criteria were i) FSH affinity, ii) cAMP production and associated transcriptional response, iii) ERK activation kinetics with or without inhibitors of cAMP/PKA pathway (H89) or β -arrestins (siRNA) and iv) ability of each receptor to recruit β -arrestins upon ligand binding. Our results showed that the R466A and T469F mutants were completely impaired in Gs-coupling whereas they both recruited β -arrestins and triggered ERK activation *via* a β -arrestin-dependent, Gs-independent mechanism.

Conclusion: Our results are consistent with the existence of several active conformations of the FSHR and show that it is possible to generate signalling-selective FSHR by mutagenesis. These selective mutants will allow us to determine the relative contributions of the β -arrestin-dependent pathway not only *in vitro* but also in the reproductive function using homologous recombination in mouse.