Biased signalling of the non-peptide luteinising hormone receptor agonist, LHR-Chap

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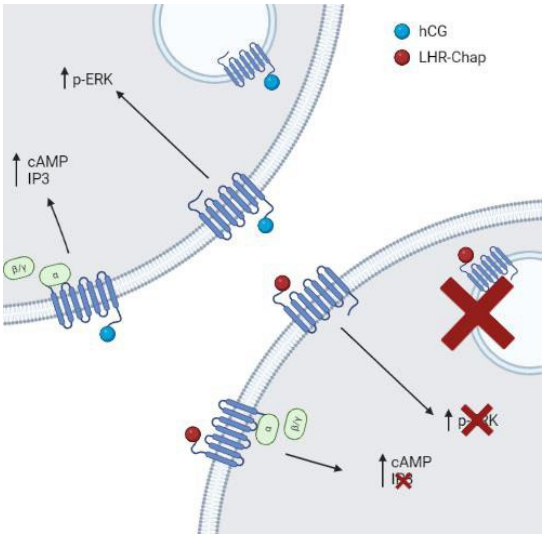

A non-peptide luteinising hormone receptor agonist, LHR-Chap, displays biased activity in cellular signalling pathways and LHR internalisation.

Introduction: The luteinising hormone receptor (LHR) and follicle-stimulating hormone receptor (FSHR) are G protein-coupled receptors (GPCRs) crucial in the endocrine control of reproduction and fertility. During assisted reproductive therapies for infertility, the gonadotropin hormones, follicle-stimulating hormone (which activates ovarian FSHRs) and luteinising hormone/human chorionic gonadotropin (hCG) (which activate ovarian LHRs), are administered to promote follicular development and oocyte ovulation, respectively. There has been much focus on development of non-peptide (small-molecule), orally-active gonadotropin replacements to improve convenience of treatment (as they can be administered orally rather than by injection) and enable more efficient and consistent synthesis (as there can be large inter- and intra-batch variation with the protein hormones). One of the most promising of the non-peptide LH/hCG replacements in development is LHR-Chap (also known as Org 42599)[1]. However, this compound has been shown to interact with the LHR at a different site to the native protein hormones.[1] Such differential interactions can result in biased activity, whereby different downstream signalling is elicited by different compounds. Therefore, the aim of this study was to investigate the similarities and differences in LHR signalling in response to activation by the native hormone (hCG) and LHR-Chap.

Methodology: Effects of hCG or LHR-Chap on G protein-dependent signalling were determined by measuring cAMP production or inositol phosphate (IP) levels via cAMP response element (CRE)-coupled luciferase assays/cAMP ELISA or [^{3}H] inositol trisphosphate (IP3) accumulation assays, respectively. Activation of G protein-independent mitogen activated protein kinase signalling pathways, was examined by Western blotting for phosphorylated extracellular signal-regulated kinase (p-ERK). Lastly, LHR internalisation, in response to stimulation, was analysed by confocal microscopy and cell surface receptor ELISA. Analyses were conducted with HEK cells transiently expressing LHRs and were confirmed in a gonadal cell line (TM3) with endogenous LHRs.

Results: cAMP stimulation was measured in response to stimulation with hCG or LHR-Chap. However, activation of IP3 production and ERK phosphorylation only occurred in response to treatment with hCG but not LHR-Chap. Prolonged stimulation with high concentrations of hCG, but not LHR-Chap, also resulted in reduced cell surface expression of the LHR, indicative of receptor internalisation (a control mechanism used to regulate agonist-mediated GPCR stimulation).

Discussion_and_Conclusion: LHR-Chap activates signalling cascades in a different manner compared to hCG which indicates ligand bias/biased signalling. Furthermore, there is evidence that LHR internalisation varies in response to treatment with hCG or LHR-Chap. Further examination of the differential signalling associated with LHR-Chap, particularly with respect to the implications for downstream physiological responses (such as sex steroid synthesis/release and ovulation), are crucial as a prelude to its future therapeutic development.

References: 1. van Koppen, C.J.et al. A signaling-selective, nanomolar potent allosteric low molecular weight agonist for the human luteinizing hormone receptor, 378 (2008) 503-514.

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