Luteinizing hormone (LH) and human choric gonadotropin (hCG) action on the same receptor results in different intracellular signaling in mouse primary Leydig cells.

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Introduction

Human luteinizing hormone (LH) and chorionic gonadotropin (hCG) are two glycoprotein hormones involved in reproduction. Despite binding the same receptor (LH/GHR), are both used in clinical practices for assisted reproduction. LH/GHR mediates specific LH/hCG-specific signaling, likely through the hinge region of the receptor, which is responsible for LH/hCG differentiation [3]. Quantitative and qualitative non-equivalence of hCG and LH was previously demonstrated in human primary granulosa cells in vitro [1].2. However, experiments in steroidogenic cells from males are missing. Since Mice Leydig cells are naturally expressing the murine LH receptor (Lhr), they are a suitable model to evaluate the action of the gonadotropins at molecular level. Although Lhr and LH/GHR doesn’t share a complete sequence identity (80%), the murine receptor retains the ability of human LH and hCG binding.

Aim

The aim of this study is to compare the LH- and hCG-mediated signaling and downstream events in mouse primary Leydig cells in vitro.

Study design

Leydig cells were collected from testis of 3/5-months-old C57BL6 mice by density gradient, then they were cultured and treated by increasing doses of recombinant LH and hCG (1 pM-100 nM range). cAMP accumulation experiments were performed in the presence of 500 μM the phosphodiesterase inhibitor IBMX, then measured by ELISA as well as testosterone synthesis. ERK1/2 and CREB phosphorylation was evaluated by Western blotting, gene expression by real-time PCR.

Results

Dose-response curves revealed that hCG is about 10-fold more potent than LH in inducing cAMP recruitment (hCG EC50 = 18.64±10.1 pm; LH EC50 = 392.0±53.96 pm; Mann-Whitney’s U-test; p<0.05; n=4), despite achieving the same plateau level at the 100 nM dose. LH and hCG stimulated the activation of ERK1/2 in the 10 pM-100 nM range over basal, moreover achieving higher levels upon hCG versus LH stimulation in the 0.1 pM-100 nM range (two-way Anova and Bonferroni post-test; p<0.05; n=5), reflecting their prevalent cAMP-dependence. LH and hCG treatments induced about equal levels of CREB phosphorylation, both resulting in a significant pCREB increase over basal between the 10-100 pM doses (two-way Anova and Bonferroni post-test; p<0.05; n=5). Downstream cell signaling-mediated events, i.e. gene expression and testosterone production, were not significantly different upon LH and hCG treatments. Indeed, the two gonadotropins induced similar Stard5 gene expression and EC50 for steroid synthesis (Mann-Whitney’s U-Test; p<0.05; n=4).

Discussion

Murine Lhr mediates a different cAMP and PKA-dependent pERK1/2 activation upon human LH and hCG treatment. However, no downstream CREB phosphorylation, gene expression and testosterone production occur, likely due to an opposite, balancing effect between cAMP and pERK1/2 on steroidogenesis. The non-human receptor mediates quantitatively but not qualitatively different LH/hCG-specific signaling, oppositely to what previously described in human primary granulosa cells. In fact, the comparison between LH/GHR and Lhr amino acid sequences revealed no identity within the hinge regions. This finding is relevant to improve the method for gonadotropin characterization based on the rat bioassy [4], which rely on the evaluation of in vivo effects mediated by non-human receptor.

References


Figure 1. Evaluation of cAMP production. Mouse primary Leydig cells were stimulated with increasing doses of hCG and LH in the presence of IBMX. Total cAMP was measured after 3 hours of incubation. Total cAMP levels normalized as percentage of the maximal response. All the results are represented as means ± SEM. In a logarithmic X-axis, non-linear regressions were plotted. The EC50 values were compared by Mann-Whitney’s U-test (p<0.05; n=4).

Figure 2. Evaluation of phospho-ERK1/2 and phospho-CREB activation in murine Leydig cells. A) The cells were stimulated for 15 min by increasing doses of hCG and LH, then pERK1/2 and pCREB signals were evaluated by Western blotting (image representative of five independent experiments). B) Semiquantitative analysis of pERK1/2 and pCREB signals. The values were normalized over the loading control total ERK (means; SEM; n=5). Significant vs control; *significant vs LH (2-way ANOVA and Bonferroni post-test; p<0.05).

Figure 3. Anatomic sequence alignment of human LH/GHR and mouse Lhr sequences (NP 080224 and NP 0381410, respectively) were obtained from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/). No color indicates amino acids residues fully conserved; green indicates conservation of strong groups; purple indicates conservation of weak groups; grey indicates conserved amino acids.

Figure 4. Steroid gene expression analysis. LH- or hCG-induced Stard5 gene expression in 12 h-stimulated mouse Leydig cells. The cells were treated using the LH and hCG EC50 doses. iRT gene expression was normalized to the mean of β-actin (mean SEM; significant versus basal; Mann-Whitney’s U-test; p<0.05; n=4).

Figure 5. Evaluation of testosterone production upon hCG or LH stimulation. Mouse primary Leydig cells were stimulated by increasing LH or hCG doses, in the presence of 500μM IBMX, and total testosterone was measured by immunoassay after 24 h. A) Testosterone levels expressed in percentage of maximal response. All the results are represented as means ± SEM. In a logarithmic X-axis, non-linear regressions were plotted. The EC50 values were compared by Mann-Whitney’s U-test (p<0.05; n=4).

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