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The liver X receptors and sterol regulatory element binding proteins alter progesterone secretion and are regulated by human chorionic gonadotropin in human luteinized granulosa cells

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ABSTRACT

There is increased expression of liver x receptor (LXR) target genes and reduced low density lipoprotein receptor (LDLR) during spontaneous luteolysis in primates. The LXRs are nuclear receptors that increase cholesterol efflux by inducing transcription of their target genes. Transcription of *LDLR* is regulated by sterol regulatory element binding proteins (SREBPs). Human chorionic gonadotropin (hCG) prevents luteolysis and stimulates progesterone synthesis via protein kinase A (PKA). Thus, our primary objectives are: 1) Determine the effects of LXR activation and SREBP inhibition on progesterone secretion and cholesterol metabolism, and 2) Determine whether hCG signaling via PKA regulates transcription of LXR and SREBP target genes in human luteinized granulosa cells. Basal and hCG-stimulated progesterone secretion was significantly decreased by the combined actions of the LXR agonist T0901317 and the SREBP inhibitor fatostatin, which was associated with reduced intracellular cholesterol storage. Expression of LXR target genes in the presence of T0901317 was significantly reduced by hCG, while hCG promoted transcriptional changes that favor LDL uptake. These effects of hCG were reversed by a specific PKA inhibitor. A third objective was to resolve a dilemma concerning LXR regulation of steroidogenic acute regulatory protein (STAR) expression in primate and non-primate steroidogenic cells. T0901317 induced *STAR* expression and progesterone synthesis in ovine, but not human cells, revealing a key difference between species in LXR regulation of luteal function. Collectively, these data support the hypothesis that LXR-induced cholesterol efflux and reduced LDL uptake via SREBP inhibition mediates luteolysis in primates, which is prevented by hCG.

1. Introduction

The mechanisms causing luteolysis of the primate corpus luteum (CL) have not been determined (Stouffer et al., 2013). It has previously been reported that there is increased expression of liver x receptor (LXR) target genes and decreased low density lipoprotein receptor (LDLR) during spontaneous luteolysis in the primate CL (Bogan and Hennebold, 2010). There are two LXR isoforms, α (NR1H3) and β (NR1H2), which are cholesterol sensors belonging to the steroid hormone receptor superfamily (Repa and Mangelsdorf, 2000). When intracellular cholesterol concentrations rise, they induce transcription of their target genes including ATP binding cassette subfamily A1 (*ABCA1*) and G1 (*ABCG1*) (Lund et al., 2003), as well as *NR1H3* itself (Laffitte et al., 2001), which results in an enhancement of cholesterol efflux. Furthermore, the LXRs inhibit uptake of LDL cholesterol by inducing transcription of myosin regulatory light chain interacting protein (*MYLIP*), which causes proteolytic degradation of LDLR (Zelcer et al., 2009). Therefore, the role of the LXRs is to reduce intracellular cholesterol concentrations.

Sterol regulatory element binding proteins (SREBPs) are important mediators of lipid metabolism (Goldstein et al., 2006). There are two genes that encode three SREBPs: sterol regulatory element binding transcription factor 1 (*SREBF1*) that encodes

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SREBP1a and SREBP1c via alternative promoters, and *SREBF2* that encodes SREBP2 (Horton et al., 2002). The SREBPs are synthesized as inactive precursors that are embedded in the endoplasmic reticulum (ER) membrane when sterol concentrations are high. When sterol concentrations fall, SREBPs are transported to the Golgi apparatus where they are proteolytically cleaved to release the active transcription factor (Goldstein et al., 2006). The SREBP1c isoform preferentially targets genes involved in fatty acid synthesis but does not increase intracellular cholesterol (Horton et al., 2002), and interestingly SREBP1c (*SREBF1*) is also a known LXR target gene (Repa et al., 2000,Yoshikawa et al., 2001). Conversely, SREBP1a and SREBP2 activate transcription of the rate-limiting enzyme in cholesterol biosynthesis 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*), as well as *LDLR*, which raises intracellular cholesterol concentrations (Horton et al., 2002). Thus, the LXRs and SREBP1a and SREBP2 have opposing actions that are necessary to maintain intracellular cholesterol homeostasis. This is further illustrated by the fact that many endogenous LXR agonists also inhibit processing of the SREBPs to their active forms (Goldstein et al., 2006,Hong and Tontonoz, 2014).

The CL is a highly steroidogenic gland that has a large demand for cholesterol (Stouffer and Hennebold, 2015). Thus, we hypothesize that limiting the intracellular cholesterol supply via increased LXR and reduced SREBP activity could reduce progesterone (P4) synthesis and cause luteolysis of the primate CL. Furthermore, it has been well established that luteolysis is prevented during early pregnancy by human chorionic gonadotropin (hCG), which binds to the luteinizing hormone/choriogonadotropin receptor (LHCGR) and maintains P4 secretion via protein kinase (PKA) (Ascoli et al., 2002). Therefore, we also hypothesize that hCG signaling via PKA inhibits LXR and stimulates SREBP target gene transcription. Correspondingly, the two primary objectives of this study are: 1) Determine the effects of LXR activation and SREBP inhibition on P4 secretion and cholesterol metabolism; and 2) Determine whether hCG signaling via PKA regulates transcription of LXR and SREBP target genes. Human luteinized granulosa cells were used for these studies as they are functionally similar to luteal cells (Stewart and Vandevoort, 1997), and express both LXR isoforms (Drouineaud et al., 2007).

The third objective was to resolve a dilemma concerning LXR regulation of steroidogenic acute regulatory protein (STAR) expression in primate and non-primate steroidogenic cells (Mouzat et al., 2013). The LXRs increase STAR expression and steroidogenesis in rodents (Manna et al., 2013,Mouzat et al., 2009), but have not been reported to induce STAR in steroidogenic primate cells (Drouineaud et al., 2007,Puttabyatappa et al., 2010). Given the critical role of STAR in steroidogenesis (Manna et al., 2016), the role of the LXRs in regulating STAR expression warrants further clarification.

2. Materials and methods

2.1. Isolation of human granulosa cells

The follicular aspirates used in this study were from 55 female patients undergoing oocyte donation or *in vitro* fertilization for male factor or idiopathic infertility at the Reproductive Health Center, Tucson, AZ. The patients were 24–44 years old at the time of retrieval. The University of Arizona Institutional Review Board approved the study and patients gave informed written consent. Follicular aspirates were centrifuged at 500 × g for 5 min at 4 °C. The supernatant was aspirated and cell pellets were suspended in nutrient mixture F10 Ham (Ham's F10) with 0.1% (*w:v*) bovine serum albumin, covered onto a 40% (*v:v*) Percoll gradient (GE Healthcare) in Hanks' balanced salt solution (Sigma-Aldrich Inc.), and centrifuged at 500 × g for 15 min at 4 °C. The supernatant was recovered and diluted with Ham's F10 and centrifuged as before. The cell pellet was washed once more with Ham's F10. Finally, the cell pellet was suspended in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DMEM/F12) with insulin-transferrin-selenium (ITS) supplement (Sigma-Aldrich) and 0.02 IU/ml hCG. Cells were counted by trypan blue (0.2% *v:v*) dye-exclusion method. Plates were coated with 5 μg/ml fibronectin at 37 °C for 1 h and washed with sterile PBS. The isolated granulosa cells were plated in 24-well plates or 96-well plates at a density of 5×10^4 cells/cm^2. Granulosa cells were cultured for 5 days in luteinization medium (DMEM/F12 with ITS supplement, 100 units/ml penicillin, 0.1 mg/ml streptomycin (Pen/Strep), and 0.02 IU/ml hCG), incubated at 37 °C and 5% CO_2 in a humidified environment, and media was changed daily.

2.2. Cell treatments

A 2 × 2 × 2 factorial design was used to test the effects of the LXR agonist T0901317 (T09, Cayman Chemical Co.), the SREBP inhibitor fatostatin (Cayman Chemical), and hCG (Fig. 1). Cells were first treated with vehicle (0.1% *v:v* DMSO), T09 (0.1 μM), and/or fatostatin (5 μM) in treatment medium (DMEM/F12 containing ITS, Pen/Strep, 20 μg/ml LDL and 10 μg/ml HDL cholesterol). Both LDL and HDL were included in treatment medium because they are necessary for cholesterol uptake and efflux, respectively. After 16 h (pretreatment period), fresh treatments with or without 2 IU/ml hCG were added for the final 4 h (challenge period).

To determine whether PKA mediates hCG effects, another 2 × 2 × 2 factorial design consisting of T09, protein kinase inhibitor 14–22 (PKI, Life Technologies, Inc.), and hCG was employed (Fig. 1). Cells were first treated with vehicle or T09 in treatment medium for 16 h. Fresh treatments were added in the presence and absence of 2 IU/ml hCG and PKI (50 μM) for the final 4 h. One additional experiment for Western blot analysis was performed. Treatment groups for this experiment included: 1) vehicle, 2) T09, 3) T09 + hCG, and 4) T09 + hCG + PKI. Treatments were applied to cells as illustrated in Fig. 1, with the exception that the final treatment in the presence and absence of hCG and PKI was extended from 4 h to 8 h to allow more time for protein turnover in response to hCG and PKI.

To determine the chronic effects of hCG, after 4 days of luteinization cells were switched to treatment medium containing 0.02 IU/ml hCG for one day. The next day cells were incubated in the presence or absence of 0.2 IU/ml hCG in treatment medium, with media changed daily. Cells were harvested every 24 h for 3 consecutive days.

2.3. Ovine mixed luteal cell isolation and treatment

Procedures involving sheep were approved by the University of

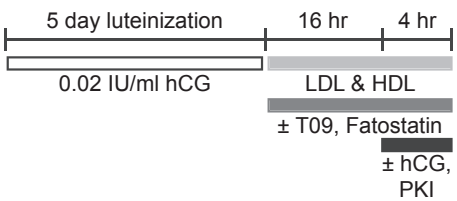

Fig. 1. Primary experimental timeline. The timeline begins with plating of luteinizing granulosa cells and ends with cell harvest. The relative timescale is shown above the line, with additives to the base medium (DMEM/F12 with ITS and Pen/Strep) indicated beneath the line.

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