

Review Article

Mechanisms of Luteinising Hormone Regulation in Female Steroidogenesis

Cadagan D*

Department of Sciences, Staffordshire University, England

*Corresponding author: Cadagan D, Lecturer in Biomedicine, Senior Biomedical Researcher, Department of Sciences, Staffordshire University, England

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Abstract

Understanding the mechanisms of steroidogenic regulation in female fertility is fundamental to determining dysfunction. Research has revealed a strong relationship between fertility and metabolic disturbances. With many endocrine signals involved in activation of steroidogenic cascades, it has become necessary to map the complex cross-talk between these regulatory systems and this review looks to establish this based on current understanding. Luteinising hormone is a vital endocrine hormone that is already known to signal *via* these pathways, although its involvement in metabolic disturbance is less understood. Studies investigating activation of the LH receptor in key steroidogenic cells, ovarian theca and granulosa, have highlighted overlaps in important signalling cascades including PKC/MAPK/PKA and PI3K. Here, we review LH and its signalling cross-talk in key steroidogenic pathways.

Keywords: Luteinising hormone (LH); Lutropin; Steroidogenesis; Gonadotropin; Ovulation; LH Receptor; Cyclic adenomonophosphate (cAMP); Diacylglycerol (DAG); Protein kinase C pathway; MAPK pathway

Introduction

The pituitary gland is responsible for secretion of various endocrine signalling hormones with the anterior pituitary producing somatotropins, thyrotropins, corticotropins, lactotropins and gonadotropins. Dysfunction of the pituitary gland can therefore have systemic effects as a result of signalling errors and conditions such as hyper and hypopituitarism. These dysfunctions have been linked to the pathophysiology of conditions such as diabetes and the metabolic syndrome. There has been a rapid increase in Diabetes Mellitus (DM) over the past decades resulting in it becoming one of the most prevalent endocrine disorders, effecting ~135 million people. Therefore, it is vital to understand the mechanisms behind the condition [1,2].

Many studies have examined the effect of diabetes on gonadotropin production with an aim of determining a relationship between impaired fertility and menstrual disturbances [3-5]. In doing so, the importance of Luteinising Hormone (LH) in pituitary regulation has been highlighted, as well as its sensitivity to metabolic changes [1]. Although reduced gonadotropin response may play only a partial role in steroidogenic function it is important to understand the mechanism and pathways involved in order to fully understand the overall dysfunction.

This review will examine our current understanding of the effects of LH on steroidogenic cells to allow future mapping of possible regulatory pathways that may be seen as targets for dysfunction in metabolic diseases.

Luteinising Hormone and Steroidogenesis

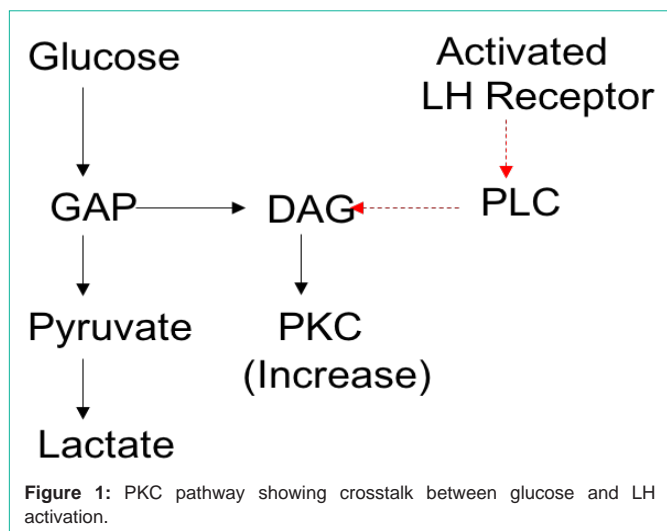
The gonadotropins are a family of proteins consisting of LH, Follicular Stimulating Hormone (FSH) and the Chorionic Gonadotropins (CG). These hormones are vital in the control and

regulation of reproductive function in males and females. LH and FSH are secreted by the anterior pituitary gland, whereas CG is produced by a component of the fertilised egg called the Syncytiotrophoblast. LH, also known as lutropin, stimulates ovulation and development of the corpus luteum females. Structurally LH is a heterodimeric glycoprotein similar in structure to FSH, thyroid stimulating hormone and Human Chorionic Gonadotropin (hCG). All four proteins share a common alpha subunit, with the beta subunits conferring biological specificity [6,7].

In females, FSH action on ovarian granulosa cells initiates follicular growth. This stimulates a rise in oestrogen and LH receptor expression. Eventual positive feedback to the hypothalamus occurs, resulting in the release of LH over a 24-48hr period. The ensuing LH surge triggers ovulation, releasing the egg and initiating the production of the corpus luteum from the remnants of the follicle. The corpus luteum secretes progesterone for endometrial preparation and the possibility of implantation. LH is maintained for up to two weeks allowing for steroidogenesis along with support *via* hCG action. In the case of pregnancy LH supports theca cells which produce androgens necessary for estradiol synthesis and cell proliferation [3,7].

LH Receptor

Luteinising hormone / chorionic gonadotropin activates multiple signal transduction systems. The luteinising hormone receptor is part of the Glycoprotein Hormone Receptor Family (GpHRs) which also includes Follicular Stimulating Hormone Receptor (FSHR) and Thyroid Stimulating Hormone Receptor (TSHR). The LH receptor differs from these through its ability to bind two glycoprotein hormones, LH and hCG. These receptors are responsible for regulation of reproductive and metabolic processes with LHR activation leading to androgen synthesis and ovulation. The GpHRs contain two major domains of approximately the same size. Firstly, a large, glycosylated



N-terminal Ectodomain (ECD) containing Leucine-Rich Repeats (LRRs) capped by cys-rich regions, the latter forming a portion of a hinge region. Secondly, a Trans-Membrane domain (TM) with seven membrane spanning helices, three extracellular loops (ecls), three intracellular loops (icls) and a short icl 4, an eighth cytoplasmic helix parallel to the plasma membrane, and a cytoplasmic tail [3]. The ECD and TM domains have important and distinct functional roles, namely hormone binding and signal transduction, respectively [8,9].

Most of the sequential steps involved after hormone binding to the ECD until G protein activation on the inner face of the plasma membrane remain poorly understood. In many experimental systems, LH or hCG binding to LHR results in activation of both protein kinase A and protein kinase C. At relatively low concentrations of LH and hCG, Gs appears to be the preferred signalling pathway, resulting in a rapid increase in the intracellular concentration of cAMP [10].

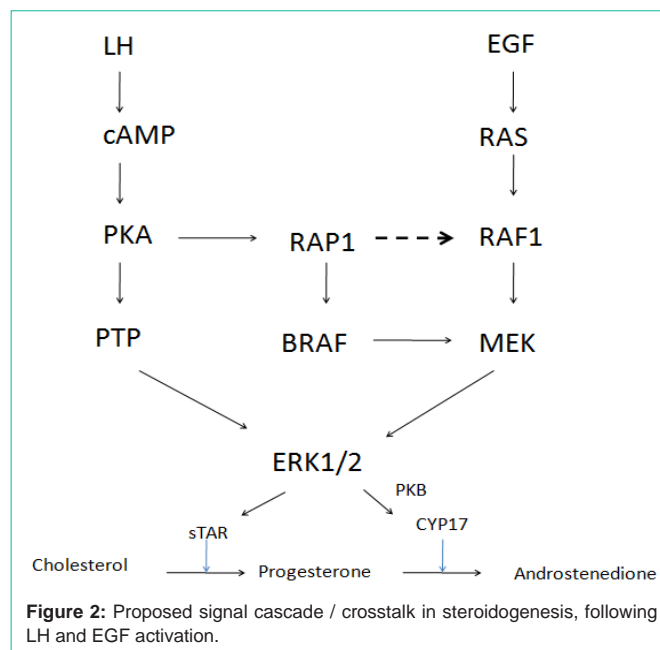
Cyclic Adenomonophosphate

cAMP is derived from adenosine triphosphate and is utilised within intracellular signal transductions as a second messenger, transferring the effects of hormones incapable of permeating cellular membranes. Typically, it allows activation of protein kinases and is also involved in the release of intracellular calcium from the endoplasmic reticulum.

cAMP synthesis occurs through adenyl cyclase action on ATP located on the inner plasma membrane and decomposition occurs through phosphodiesterase action on cAMP. This action occurs through stimulatory G-protein (Gs)-coupled receptor activation and is inhibited by agonists of adenylyl cyclase inhibitory G (Gi)-protein-coupled receptors.

Adenylyl cyclase can be activated or inhibited by G proteins, which are coupled to membrane receptors and thus can respond to hormonal or other stimuli. Following activation cAMP acts as a second messenger by interacting with and regulating other proteins such as protein kinase A and cyclic nucleotide-gated ion channels [11,12].

Studies in pre-pubertal female rats have shown that in conditions such as uraemia can lead to reduced gonadotropin - cAMP sensitivity.



This condition is related to metabolic disturbance and has direct associations to diabetes mellitus and diabetes induced cardiac failure as well as chronic kidney failure [13]. Furthermore, female uremic patients have shown menstrual irregularities and cases of delayed puberty possibly associated with gonadotropin target response. However, research in uremic premenopausal women has also shown hormonal disturbances including an absence in pre-ovulatory peaks of LH and estradiol concentrations. The source of these irregularities may therefore originate within the hypothalamus *via* impaired production of GnRH or in the anterior pituitary rather than within the cellular targets [14].

Diacylglycerol / Protein Kinase C Pathway

Diacylglycerol (DAG) is a key second messenger that regulates important cellular responses including proliferation, apoptosis, immune response and differentiation. This occurs *via* its target protein Protein Kinase C (PKC). Activation is linked to the LH receptor as a hepta-membrane spanning protein that couples stimulatory binding proteins Gs and Gq to allow adenylyl cyclase signalling and increases in cAMP. This subsequently activates cAMP dependent PKA [11,12].

LH receptor activation increases intracellular calcium as a result of Phospholipase C activation (PLC) [5,15]. Once activated PLC allows cleavage of phosphatidylinositol, 4,5-biphosphate into inositol-1,4,5-triphosphate (IP3) and Diacylglycerol (DAG). IP3 then binds to intracellular calcium channels on the endoplasmic reticulum resulting in increased cytosolic calcium levels. DAG binds to Protein Kinase C (PKC) allowing co-activation (Figure 1) [16,17]. PKC requires calcium, phosphatitylserine and DAG for activation. These regulators allow a conformational change revealing the substrate binding site [18].

Mitogen Activating Protein Kinase (MAPK) activation has been found to be independent of PKC activation in ovarian granulosa cells. This suggests an independent pathway that maybe regulated by LH alone. Furthermore, PKC has been seen to be partially activated

by glucose and GAP in the absence of LH receptor activation in granulosa cells which may allow for pathway cross talk [19].

MAPK Pathway

Ras proteins are ubiquitous GTPases located on the inner surface of plasma membranes. They communicate receptor-mediated signal transduction pathways, transmitting extracellular signals that promote growth, proliferation, and differentiation. The signalling cascade starts from the plasma membrane where the growth factor binds to its enzyme-linked receptor. Receptor-linked tyrosine kinases such as the Epidermal Growth Factor Receptor (EGFR) are activated by extracellular ligands and binding of EGF's. This leads to phosphorylation of the intracellular parts of the receptors. Activating Guanine Exchange Factors (GEFs), such as SOS and GEFs allow the exchange of GDP to GTP activating Ras [16]. The major downstream target of Ras-GTP is Mitogen-Activated Protein Kinase (MAPK), but also activates phosphatidylinositol 3-kinase (PI3-kinase), Ras-related guanine nucleotide dissociation stimulator (RalGDS). Activation of MAPK occurs through specific phosphorylation of Raf, which is activated on the plasma membrane by Ras-GTP. Raf phosphorylates Mitogen-Activated Kinase 1/2 (MEK1/2 kinase), in turn activating the extracellular regulated kinase 1/2 (ERK1/2 kinase or p44/42 MAPK) by phosphorylation.

The second class of enzymes in the ERK cascade are the Mitogen-Activated Protein Kinases (MEKs). These phosphorylate threonine and tyrosine residues. MEK1 and MEK2 are involved in ERK signalling and activated *via* there only substrate, Raf kinases. Inactivation of the ERKs can occur through de-phosphorylation *via* serine threonine or tyrosine phosphatases [20,21].

ERK1/2 kinase phosphorylates a variety of downstream targets, which results in changes in gene expression and the catalytic activities of enzymes and makes them important targets in steroidogenic activity [22].

A recent study showed that both MAPK kinase (MEK1/2) phosphorylation and extracellular signal related kinase (ERK1/2) phosphorylation were reduced by 50% in Polycystic Ovarian Syndrome (PCOS) steroidogenic cells compared with that in normal cells. A reduction in MEK1/2 and ERK1/2 phosphorylation is associated with increased theca CYP17 gene expression and subsequent androgen biosynthesis [23].

Treatment of ovarian steroidogenic theca cells with the MEK inhibitor PD98059 has been shown to augment theca CYP17 gene expression and androgen biosynthesis [23]. The extent to which the other components of the MAPK pathway contribute to overall androgen biosynthesis requires further examination.

Although LH has been reported to activate the cAMP/PKA pathway and the ERK/MAPK pathway in theca cells, whether and how LH stimulates the PI3K/Akt cascade in theca cells remains unclear. Recent studies have shown LH stimulates Akt phosphorylation in both cultured bovine and human theca cells, and that activation of PI3K/Akt is involved in CYP17A1 mRNA expression and androgen production in theca cells thus supporting LH activation of PI3K [24-26]. H89, a potent and selective inhibitor of PKA, did not affect LH-mediated changes in phospho-Akt, indicating that a pathway distinct from that of the PKA is involved in LH-induced Akt phosphorylation

in theca cells.

In contrast to PKA inhibitor, the MEK inhibitor (U0126) blocked LH-mediated Akt phosphorylation and androgen production in theca cells. This suggests that LH stimulates CYP17A1 mRNA expression and androgen production in theca cells *via* activation of the PI3K/Akt pathway and with the involvement of the MAPK pathway.

While the precise mechanism for the activation of PI3K pathway by LH in theca cells is not known, we propose LH-induced phospho-Akt up-regulation may involve MAPK-mediated down-regulation of phosphatase and tensin homologue (PTEN) a tumour suppressor which negatively regulates Akt phosphorylation. In this context, it has been shown that PI3K is required for estradiol-stimulated hepatic cell growth and that the MAPK pathway reduces the level of PTEN, allowing estradiol-induced phosphorylation of Akt [27]. Whether this indeed is the case in the theca cells awaits further investigation.

Discussion

Clarification of the LH-mediated intracellular signalling events is essential along with consideration for cross talk and compensatory mechanisms between pathways. This will allow for better understanding of not only ovarian physiology, but also of the pathophysiology of steroidogenic dysfunction.

Studies in ovarian steroidogenic granulosa cells have shown elevated cAMP and activated ERK. PKA forms inactive RAP1 inhibiting RAF1 and preventing MEK phosphorylation of ERK and subsequent androgen synthesis [28]. Based on these findings increased cAMP may allow phosphorylation of cAMP-responsive guanine nucleotide exchange factors (Epac1 and Epac2). Upon binding of cAMP, Epac1/2 may rapidly activate Rap1, which subsequently promotes activation of B-raf and the rest of the ERK cascade [29].

Direct activation by cAMP, RAP1 and subsequent BRAF activation would lead to MEK phosphorylation (LH mediated androgen synthesis). Inhibition of PKA would prevent RAP1 formation and eventual RAF1 inhibition would return due to reduced RAP1 formation (Figure 2). This is supported by studies showing LH mediated ERK phosphorylation only occurring within 24hrs of LH exposure during PKA inhibition [30].

With the importance of steroidogenic regulation in fertility and our understanding of the relationship this may have with metabolic dysfunction, it is important to understand cross talk within regulatory pathways and the involvement of hormonal regulators. LH continues to show a vital role in multiple signalling cascades and its specific involvement requires further examination.

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