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Original Research Article

Thecal cell sensitivity to luteinizing hormone and insulin in polycystic ovarian syndrome

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ARTICLE INFO

Article history:
Received 14 May 2015
Received in revised form
6 December 2015
Accepted 30 December 2015
Available online 13 January 2016

Keywords:
17α-Hydroxylase
Androgen
Insulin
Luteinizing hormone
Theca

ABSTRACT

This study examined whether a defect of steroid synthesis in ovarian theca cells may lead to the development of PCOS, through contributions to excess androgen secretion.

Polycystic ovarian syndrome (PCOS) is one of the leading causes of infertility worldwide affecting around 1 in 10 of women of a reproductive age. One of the fundamental abnormalities in this syndrome is the presence of hormonal irregularities, including hyperandrogenemia, hyperinsulinemia and hypersecretion of luteinizing hormone (LH). Studies suggest that insulin treatment increases progesterone and androstenedione secretion in PCOS theca cells when compared to insulin treated normal theca cells. Furthermore the augmented effects of LH and insulin have been seen to increase ovarian androgen synthesis in non-PCOS theca cultures whilst also increasing the expression of steroidogenic enzymes specific to the PI3-K pathway.

Our examination of primary thecal cultures showed an increase in both the expression of the steroidogenic enzyme CYP17 and androgen secretion in PCOS theca cells under basal conditions, when compared to non-PCOS cells. This was increased significantly under treatments of LH and insulin combined.

Our results support the previous reported hypothesis that a dysfunction may exist within the PI3-K pathway. Specifically, that sensitivity exists to physiological symptoms including hyperinsulinemia and hyper secretion of LH found in PCOS through co-stimulation. The impact of these findings may allow the development of a therapeutic target in PCOS.

1. Introduction

Polycystic ovarian syndrome (PCOS) is a disorder of diverse clinical and biochemical symptoms including anovulation and hyperinsulinemia [1–3]. Typically PCOS is associated with high circulatory luteinizing hormone (LH) and testosterone levels [4,5]. Currently evidence suggests that theca cell androgen secretion contributes to hyperandrogenemia in PCOS [6,7], however it is not clear whether this is through thecal hypertrophy or dysfunction.

Steroidogenic activity within the ovaries is dependent on the collaboration of granulosa and theca cells. Androgen biosynthesis occurs predominantly within the ovarian theca

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http://dx.doi.org/10.1016/j.repbio.2015.12.006

cells and contributes to around 50% of overall physiological androstenedione secretion [8]. The excess androgen synthesis found within PCOS is thought to relate to excess ovarian biosynthesis and although the exact underlying etiology of PCOS remains unexplained, significant evidence suggests that an excess of ovarian androgen production is central to the condition [3,9]. Three possible mechanisms for androgen hyper-secretion have been proposed. These include: an intrinsic functional thecal defect, hyperinsulinemia resulting from insulin resistance, or pituitary LH hyper secretion resulting in excessive thecal stimulation. Several publications exist showing the effects of insulin on thecal function in normal and polycystic ovaries [10–12]. However combined influence of both insulin and LH is yet to be examined in PCOS, and as both hormones are increased in PCOS, understanding their influence and cellular mechanism is of great importance.

Despite the prevalence of insulin resistance in women with PCOS [13], several studies have shown insulin receptors to be normal in structure [14], quantity and insulin binding affinity [15]. It is possible that insulin resistance in PCOS is due to post-receptor defects in the insulin-signaling pathway. It has been clearly shown that insulin acts on theca cells through its own receptor [16,17], and that despite insulin resistance in women with PCOS, ovarian theca cells show hypersensitivity to insulin resulting in excess androgen production. One explanation for this paradox may be that selective defects of insulin sensitivity exist which affects metabolic, but not mitogenic insulin signaling pathways [18,19]. Alternatively insulin sensitivity maybe tissue-specific with resistance in peripheral (skeletal and adipose) tissues and hypersensitivity in ovarian theca cells [20]. In support of this a study by Yen et al., has shown cell-specific alterations in insulin receptor substrate (IRS) protein concentrations in theca cells from polycystic ovaries that are consistent with an exaggerated amplification of the insulin signal [21].

Another area of uncertainty is whether insulin stimulates ovarian steroidogenesis independently or through augmentation of LH stimulated androgen synthesis. Nestler et al. [17] demonstrated that insulin alone stimulated a 4–13 fold increase in testosterone production by cultured theca cells from normal and polycystic ovaries. In contrast Munir et al. [16] found that insulin alone was not able to stimulate 17α-hydroxylase activities in cultured theca cells from normal ovaries, but required forskolin-mediated activation of cAMP.

In addition to these studies, Gilling-Smith et al. [22] examined androstenedione secretion in theca cells from non-PCOS and PCOS patients under varied LH treatments. They found significant increase in androstenedione secretion from PCOS theca cells both at baseline and after treating with LH. A combined effect of LH and insulin on androstenedione secretion is yet to be examined in PCOS theca cells. However, all these data point to variations within the PCOS thecal cell steroidogenic pathway. More recently, Munir et al. [16] found that inhibition of the PI3-K pathway but not MAPK, caused a decrease in insulin-forskolin driven CYP17 expression in non-PCOS theca cultures. This suggests an important role for LH in the thecal PI3-K pathway.

Clinical studies by Erickson et al. [6] suggested that the key to PCOS hormonal dysregulation is related to the steroidogenic enzyme CYP17. Furthermore, results from Munir et al. [16] showed that expression of CYP17 is increased following augmentation of insulin and LH in non-PCOS thecal cultures [23].

We therefore sought to examine the effects of insulin and LH on PCOS theca cell CYP17 expression and androstenedione secretion; and whether this varied from that shown in non-PCOS theca cells.

2. Materials and methods

2.1. *Patients, tissue collection and primary culture development*

Ovarian specimens were collected from participating women during a planned surgical procedure in the Department of Obstetrics and Gynaecology, of the Royal Derby Hospital. Recruitment was based on specific patient criteria and following informed, written consent. All women were of childbearing age, ranging from 20–45 years with BMI $<35\ kg/m^2$. Study group included women with PCOS ($n = 3$) diagnosed according to Rotterdam criteria (two of three features: anovulation, hyperandrogenemia and, polycystic ovaries on ultrasound). Control group included healthy women ($n = 3$) with regular menstrual cycles, normal serum levels of androgen (0.2–2.9 nmol/l) and fasting insulin (17.8–173 pmol/l). Exclusion criteria consisted of any metabolic or endocrine disease, such as diabetes mellitus and thyroid disease, concurrent treatment with hormonal therapy such as hormonal contraception, progestogen therapy, thyroxin hormone or corticosteroids, metformin or cholesterol lowering agents. All materials were supplied by Sigma–Aldrich Company Ltd, Dorset, UK unless otherwise stated. Biopsies were collected in Hanks Balanced 1% penicillin (100 U/mL), streptomycin (100 U/mL), HEPES (15 mM) and transported to the lab. Only small antral follicles <5 mm in diameter were used for the study. Such individual follicles (not pooled) were hemisected and the exposed theca interna, observed were then micro dissected and enzymatically dispersed using collagenase (0.5 mg/mL), collagenase IA (0.5 mg/mL), and deoxyribonuclease (0.1 mg/mL) (Sigma, UK) in HEPES-buffered DMEM containing 4.5 g/l D-glucose supplemented with 10% (v/v) fetal bovine serum (FBS) and antimicrobial agents (200 IU/mL penicillin, 200 μg/mL streptomycin, 0.50 μg/mL amphotericin B, and 100 μg/mL gentamycin) based on published methods Munir et al. [16] Mcallister et al. [24] Gilling-Smith et al. [22]. The isolated theca cells were then grown to confluence in a humidified environment of 5%/CO2/air and passaged as required (2–4).

2.2. *Assay design*

Following passage of primary cultured cells at passages of 3–5, the viability of the cells was determined using 1:1 (v/v) trypan blue and cells suspended in media composed of DMEM/F12 modified, with 15 mM HEPES, 2.5 mM L-glutamine, pyridoxine HCl, 55 mg/l sodium pyruvate, 1% penicillin (100 U/mL), streptomycin (100 U/mL), selenium (20 nM), insulin (20 nM), FBS 10% (v/v), 2% UltroSer G serum (Pall France)[24]. Theca cells were adjusted to a final cell concentration of 10^4 cells/mL and