Steroids 75 (2010) 810–817

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

The conversion of dehydroepiandrosterone into androst-5-ene-3β,17β-diol (androstenediol) is increased in endometria from untreated women with polycystic ovarian syndrome

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ARTICLE INFO

Article history:
Received 29 January 2010
Received in revised form 1 April 2010
Accepted 22 June 2010
Available online 8 July 2010

Keywords:
PCOS
Endometrium
Androstenediol
Dehydroepiandrosterone
Intracrinology
Steroid metabolism

ABSTRACT

The changes in endometrial homeostasis found in women with polycystic ovarian syndrome (PCOS) could be associated with alterations in the intracrine metabolism of steroid hormones. The uptake of dehydroepiandrosterone-sulphate (DHEA-S), precursor of the intracrine pathway, is achieved by transporters, such as organic anion transporter polypeptides (OATPs), and molecules with oestrogenic activity, such as androst-5-ene-3β,17β-diol (androstenediol), can be generated. We aimed to determine androstenediol generation and the expression of OATPs in human endometria throughout the menstrual cycle and in endometria from PCOS women. Endometrial samples were obtained from control women in the proliferative phase (control endometria (CEp), $n = 7$), secretory phase (CEs, $n = 7$), and from PCOS patients (PCOSEp, $n = 7$). The mRNA levels of OATP-B, OATP-D and OATP-E were measured by reverse transcriptase polymerase chain reaction (RT-PCR) and protein levels of OATP-E by immunofluorescence; 3β-hydroxysteroid dehydrogenase (HSD) by immunohistochemistry/Western blot; the metabolism of DHEA to androstenediol was evaluated by thin layer chromatography–high-performance liquid chromatography (TLC–HPLC). Lower levels of OATP-E transcript were obtained in PCOSEp ($p < 0.05$) compared with CEp, while OATP-E protein levels ($p < 0.05$) and DHEA conversion to androstenediol ($p < 0.01$) were higher in PCOSEp. Lower 3β-(hydroxysteroid dehydrogenase) HSD protein levels were found in PCOSEp ($p < 0.05$) (Western blot, immunohistochemistry). These results reveal a higher capacity of the endometria from PCOS women to metabolise DHEA to androstenediol, which, coupled with the high oestrogen sensitivity previously found in these endometria, may account for the increase in cell proliferation in PCOSEp already reported.

During the proliferative phase of the menstrual cycle, a predominance of the action of 17β oestradiol of ovarian origin is observed, which induces cell proliferation and growth of endometrial tissue [1,2]. In polycystic ovary syndrome (PCOS), a large percentage of women are anovulatory; therefore, their endometria are maintained at the stage of the proliferative phase [2]. PCOS is an endocrine-metabolic disorder that affects between 5% and 10% of women of childbearing age [3]. Currently, the diagnosis of this syndrome is based upon signs of clinical or biochemical hyperandrogenism and, also, if the patient presents oligo-ovulation or anovulation, and/or polycystic ovaries at ultrasonography [4,5].

Women with PCOS exhibit a greater plasma level of dehydroepiandrosterone-sulphate (DHEA-S) [6,7], infertility, ovarian dysfunction and insulin resistance with compensatory hyperinsulinaemia [3,8]. In addition, these women have a higher rate of recurrent abortions, hyperplasia and endometrial adenocarcinoma than control women [8–11]. In previous studies, we have described a high expression of oestrogen and androgen receptors and co-activators of steroid receptors, such as, AIB1 and ARA70 in the endometrium of women with PCOS, besides an increase in the intracellular steroid metabolism, all of which can be interpreted as an increased sensitivity to steroidal action in these endometria [12,13].

The intracrinology is defined as the ability of a peripheral non-steroidogenic cell to synthesise steroid hormones, which elicits their effect on the same cell. The precursor of these steroids is DHEA, synthesised and subsequently secreted from the adrenal gland into the circulation. Importantly, a number of peripheral organs,

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0039-128X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2010.06.011

Table 1
Primer sequences for PCR of cDNA for the analysis of organic anion transporter polypeptide B (OATP-B), organic anion transporter polypeptide D (OATP-D), organic anion transporter polypeptide E (OATP-E) and rRNA 18s.

Gene	Primers	Sequence	PCR product size (bp)
OATP-B	Sense	5′-CAT GGG ACC CAG GAT AGG GCC AGC G-3′	718
	Antisense	5′-GGC CTG GCC CCA TCA TGG TCA CTG-3′	
OATP-D	Sense	5′-GCT GAG AAC GCA ACC GTG GTT CC-3′	163
	Antisense	5′-GAC TTG AGT TCA GGG CTG ACT GTC C-3′	
OATP-E	Sense	5′-GCC ATG CCA CTG CAG GGA AAT G-3′	291
	Antisense	5′-TTC TGG TAC ACC AAG CAG GAG CCC-3′	
rRNA 18S	Sense	5′-GTA ACC CGT TGA ACC CCA TT-3′	200
	Antisense	5′-CCA TCC AAT CGG TAG TAG CG-3′	

through their intracellular enzymatic machinery, are capable of transforming precursors to steroids with androgenic or oestrogenic activity [14–16].

DHEA enters the cell from circulation by passive diffusion; by contrast, its sulphated form needs special transporters such as some organic anion transporter polypeptides (OATPs). Many OATPs have been identified in various tissues, and the studies have been conducted mainly in kidney, placenta, brain and liver [17]. In fact, the mammary gland expresses transporter OATP-B and, to a lesser extent, OATP-D and OATP-E [18]. Furthermore, in the intestinal cell line Caco-2, it was observed that the transport capacity of oestrone 3-sulphate into the cell by OATP-B is higher compared with the other two transporters [19]. In human endometrium, the expression of these transporters has not been detected; however, the protein levels of OATP-D have been determined in rat endometrial tissue [20].

Once inside the cells, DHEA can be metabolised and converted into androstenediol by the activity of the enzyme 17β hydroxysteroid dehydrogenase (17β-HSD), which in turn can be converted into testosterone by the activity of the enzyme 3β-HSD [21–25]. Androstenediol has the ability to induce oestrogenic or androgenic activity by binding to their receptors, having a role in the proliferation of pathological tissues such as breast and prostate cancer, without the need to be metabolise to other steroids [23,26–29]. Androstenediol has less affinity for the androgen receptor than testosterone and dihydrotestosterone (DHT); on the other hand, ligands of oestrogen receptors such as oestradiol, oestrone and oestriol have more affinity than androstenediol [26,31,32]. Thus, the activity of androstenediol depends on the concentration of oestradiol, since high concentrations of 17β-oestradiol induce androstenediol to bind to androgen receptors, whereas a lower concentration of 17β-oestradiol enhances the binding of androstenediol to oestrogen receptors, as reported for breast cancer cells [29,30]. Therefore, in the present investigation, we aimed to study the transporter proteins involved in the entry of sulphated steroids to cells and the conversion of DHEA into androstenediol in control endometria during the menstrual cycle and whether in the PCOS endometria these parameters are modified.

1. Experimental

This investigation was approved by the University of Chile Clinical Hospital and School of Medicine, University of Chile Ethical Committees and informed written consent was obtained from all subjects, in agreement with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki).

1.1. Reagents

The polyclonal antibodies for OATP-E and 3β-HSD were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The monoclonal antibody for β-actin was purchased from Sigma (MO, USA). The secondary antibody for goat-fluorescein isothiacyanate (FITC), mouse-horseradish peroxidase (HRP) and goat-HRP were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), Amersham Biosciences (Piscataway, NJ, USA) and KPL (Gaithersburg, MD, USA), respectively. The Moloney-murine leukaemia virus (M-MLV) reverse transcriptase was obtained from Invitrogen (CA, USA); Taq DNA polymerase from Biotools (Madrid, Spain); diaminobenzidine (DAB) from DAKO (Carpinteria, CA, USA); BCA Protein Assay Kit from Pierce (IL, USA); β-nicotinamide adenine dinucleotide phosphate, reduced tetra (cyclohexylammonium) salt from Sigma (MO, USA); the TRIzol reagent from Invitrogen (CA, USA) and the Western blotting (WB) substrate from Pierce (IL, USA).

1.2. Subjects

The endometrial specimens ($n = 21$) were classified as follows: endometria obtained during the proliferative phase of the menstrual cycle (control endometria (CEp), $n = 7$), endometria obtained during the secretory phase of the menstrual cycle (CEs, $n = 7$) and endometria obtained from patients with PCOS (PCOSEp, $n = 7$). The tissues were obtained with a pipelle suction curette from the uterine corpus from women with PCOS. The diagnosis of PCOS was made according to the Rötterdam Consensus [4] and Excess Androgen Society [5], considering clinical and/or biochemical signs of hyperandrogenism and at least one of two other criteria: oligo- and/or anovulation and polycystic ovaries. Hyperprolactinaemia (prolactin (PRL) > 35 ng ml^{-1}), androgen-secreting tumours (total testosterone > 2 ng ml^{-1}; DHEA-S > 3600 μg ml^{-1}), Cushing's syndrome (urine cortisol concentration > 150 μg 24 h and fasting plasma concentration of cortisol > 25 μg dl^{-1}), congenital adrenal hyperplasia (17-OH progesterone > 2.5 ng ml^{-1}), attenuated 21-hydroxylase deficiency, diabetes and thyroid disease (thyroid stimulating hormone (TSH) > 5 U/l^{-1}) were excluded. Glucose and insulin levels were evaluated by an oral glucose tolerance test; we measured plasma glucose and insulin levels at 2 h post-load of glucose. Then, the diagnosis of hyperinsulinaemia was determined when levels of insulin were 2 SD (standard deviations) of insulin concentration over the mean of the control group, as in previous studies [33]. All women included in the study had normal glycaemia values during oral tolerance glucose test and the women with PCOS presented hyperinsulinaemia. Endometria from patients without PCOS (control endometria, CE) were obtained by hysterectomy from women with non-neoplasic pathology at hysterectomy. None of the women (control or PCOS) had received hormonal therapy, clomiphene citrate or insulin sensitisers within 3 months prior to recruitment into the study, and the endometria used in this study all showed normal morphology. The endometrial phases were evaluated by an experienced pathologist using the histological criteria of Noyes [34].