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Research paper

Elevated expression of steroidogenesis pathway genes; CYP17, GATA6 and StAR in prenatally androgenized rats

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ABSTRACT

It is believed that excess androgen exposure of the fetus, via altered gene expression, causes hyperandrogenism a key feature of polycystic ovary syndrome (PCOS). The aim of this study was to evaluate expression of Cytochrome P450-17 (CYP17), GATA-binding protein (GAGT6) and Steroidogenic acute regulatory protein (StAR), genes of adult female rats prenatally exposed to androgen excess, closely reflect endocrine and ovarian disturbances of PCOS in women, by comparing them during different phases of estrus cycle with those of non-treated rats. Both the adult prenatally testosterone exposed and control rats (n = 23, each) were divided into four groups based on their observed vaginal smear (proestrus, estrus, metestrus and diestrus) and the relative expression of CYP17, GATA6 and StAR genes was measured in ovarian theca cells using Cyber-green Real-Time PCR. Serum sex steroid hormones and gonadotropins levels were measured using the ELISA method; a comparison of these two groups showed that there was an overall increase in the studied genes (CYP17; 2.39 fold change, 95% CI: 1.23–3.55; $P < 0.05$, GATA6; 2.08 fold change, 95% CI: 1.62–2.55; $P < 0.0001$, and StAR; 1.4 fold change, 95% CI: 1.02–1.78; $P < 0.05$), despite variations in different phases with maximum elevation for all genes in diestrus. The changes observed may impair the normal development of ovaries that mediate the programming of adult PCOS.

1. Introduction

Polycystic ovarian syndrome (PCOS), a complex hormonal disorder involving the reproductive and metabolic systems (Silva Dantas et al., 2013), affects 15%–20% of women in reproductive age (Sirmans and Pate, 2013). The syndrome is characterized by chronic anovulation and hyperandrogenism in the absence of specific diseases of the ovaries, adrenals, and the pituitary gland (Dunaif and Thomas, 2001). Based on familial clustering and studies on the prevalence of syndrome in twins, interaction of genetic and environmental factors in the pathogenesis of PCOS has been widely accepted (Lunde et al., 1989; Diamanti-Kandarakis et al., 2006; Vink et al., 2006).

Given the clinical heterogeneity and complex nature of the syndrome, development of animal models is the best approach to understand pathophysiologic mechanisms associated with the early etiology of PCOS that are difficult to resolve from human studies.

Emerging research findings confirm the role of excess fetal androgen as a main environmental factor, in development of the syndrome in adulthood (Barnes et al., 1994; Davies and Norman, 2002; Abbott et al., 2007). Therefore, several animal models have been developed in an attempt to understand the potential contribution of exposure to excess steroids on the development of this syndrome (Abbott et al., 2008; Walters et al., 2012). Useful animal model, should ideally replicate key features associated with human PCOS.

Ovarian hyperandrogenism is a key feature of PCOS (Carmina, 2002). PCOS theca cells showing remarkable increased androgen biosynthesis and elevated levels for expression of steroidogenic enzymes, including

Abbreviations: PCOS, polycystic ovary syndrome; CYP17, Cytochrome P450-17; GATA6, GATA-binding protein; StAR, Steroidogenic acute regulatory protein; RIES, Research Institute for Endocrine Sciences; PNA, Prenatally Androgenized; T, Testosterone; LH, Luteinizing Hormone; FSH, Follicle-Stimulating Hormone; E, Estrogen; P, Progesterone; 3′-UTR, three prime Untranslated Region; HPG axis, the Hypothalamic–Pituitary–Gonadal axis; CI, Confidence Interval; ELISA, Enzyme-Linked Immunosorbent Assay; DHEA, Dehydroepiandrosterone/Dehydroepiandrostenedione.

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Table 1
Sequences of the primers.

Gene symbol	Gene name	Sequence (5′ → 3′)
CYP17A	Cytochrome P450-17	F: AGAATTCTCTGGTCGGCC R: TTCTCCAGTTTCTGGCCA
GATA6	GATA-binding protein 6	F: GCCCCTCATCAAGCCACA R: CATAGCAAGTGGTCGAGGCA
StAR	Steroidogenic acute regulatory protein	F: CTCAACAACCAGGAAGGCT R: ATAGAGTCTGTCCATGGGCT
B-act	Beta-actin	F:CCGTGAAAAGATGACCCAGATC R: CACAGCCTGGATGGCTACGT

Cytochrome P450-17 (CYP17) and GATA-binding protein (GAGT6) (Nelson et al., 1999; Wood et al., 2003).

CYP17 enzyme mediates the conversion of pregnenolone or progesterone to 17α-OH pregnenolone or 17α-OH progesterone by 17α-hydroxylation, then cleavages the c17, 20 bonds of these to produce androgens DHEA or androstenedione in rodent gonads and androgens and corticosteroids in human gonads and adrenals (Miller, 2002; Rainey et al., 2002). The other function of CYP17 is 17, 20-lyase activity (Hall, 1986). GATA-6, as one of the six members of evolutionarily conserved GATA family of transcription factors, plays crucial roles in the development and differentiation of all eukaryotic organisms in vertebrates (Viger et al., 2008), accompanied by GATAs 4 and 5, mainly found in tissues of mesodermal and endodermal origin (heart, gut, and gonads) (Molkentin, 2000). Since, GATA6 specifically, is found in both post-mitotic germ cells and the ovarian epithelium of fetal rat ovary (LaVoie et al., 2004), it may have a role in primary ovarian morphogenesis, it also enhances transcription of steroid-metabolizing enzymes required for producing DHEA and the expression of enzymes needed to produce adrenal androgens in the adrenal (Jimenez et al., 2003). The transfer of cholesterol from the outer to the inner mitochondrial membrane is the rate-limiting step in hormone-induced steroid formation, is facilitated by mediators such as the steroidogenic acute regulatory (StAR) protein (Rone et al., 2009). StAR, as a member of the high-affinity lipid and sterol carriers family (Stocco, 2001), is primarily present in all tissues producing steroids, including the adrenal cortex, gonads, brain and the nonhuman placenta (Bhangoo et al., 2006).

We have previously introduced a rat model for PCOS which convincingly resembling endocrine and ovarian disturbances similar to PCOS, with minimal morphological disorders in the reproductive system in response to a single dose of testosterone on gestational day 20 (Tehrani et al., 2014). Different aspects of the animal model have been described elsewhere (Daneshian et al., 2015; Noroozzadeh et al., 2015). In this study, we examined whether this prenatal androgen excess alters the expression of the aforementioned critical genes, involved in the steroidogenesis pathway that may lead to development of polycystic ovary syndrome.

2. Materials and methods

2.1. Animals

Animals were handled in accordance with the ethical principles of laboratory animal care. This study approved by the local ethics committee of the Research Institute for Endocrine Sciences (RIES; 320 EC 90.09.07). Female adult Wistar rats (n = 16, age 75–95 days and body weight 170–180 g) were obtained from the RIES animal facility of the Shahid Beheshti University of Medical Sciences (Tehran, Iran) and Pasteur Institute, Karaj Production Center (Karaj, Iran). One male and one female rat were kept in a polypropylene cage (43 cm × 30 cm × 15 cm) overnight in standard animal housing conditions (12 h–12 h light–dark cycles and controlled temperature of 22 ± 3 °C, relative humidity 45–55%). The first day of pregnancy was estimated by observation of a vaginal plug after mating. Pregnant rats were randomly divided into two groups; the experimental and the vehicle (control) groups (n = 8 each). Female pups were kept for later experiments.

2.2. Hormonal treatment

Pregnant rats of the experimental group (n = 8) received 5 mg of free testosterone (T1500; Sigma, Steinheim, Germany) dissolved in a 500 μl cocktail containing sesame oil (S3547; Sigma, Steinheim, Germany) and benzyl benzoate (B6630; Sigma, Steinheim, Germany) at a ratio of 4:1, by s.c. injection on the 20th day of pregnancy, while the controls (n = 8) received only 500 μl of solvent (Tehrani et al., 2014).

Female offspring of the experimental group (prenatally androgenized, PNA) and control (n = 23, each) were kept with ad libitum food and water; the development and functioning of their reproductive system were examined between 100 and 110 days of age (in adulthood), and the estrous cycle was monitored between 70 and 90 days of age.

2.3. Determination of estrous cycle (vaginal smear)

Based on the protocol of a previous study (Tehrani et al., 2014), the estrous cyclicity was monitored by observation of vaginal smears for all androgenized female offspring (age 70–77 days). To avoid neural stimulations and stress, this was performed after deep anesthesia by IP injection of pentobarbital sodium (P3761, Sigma, St Louis, MO, USA) dissolved in normal saline 0.9% [60 mg (kg body weight)$^{-1}$], before blood and tissue sampling. After determination of estrus cycle phase based on vaginal smear observations, the animals were divided in to four sub-groups, i.e., proestrus, estrus, metestrus and diestrus.

2.4. Blood sampling

Blood samples for hormonal measurement were obtained from the abdominal aorta of the adult control and prenatally androgenized

Table 2
Comparison of sex steroid hormones levels in the studied groups. Data are presented as mean ± SEM.

Group		Testosterone		Estrogen		Progesterone	
		ng/mL	P-value	ng/mL	P-value	ng/mL	P-value
Proestrus	PNA (n = 6)	2.46 ± 0.16	0.026	96.86 ± 29.62	0. 898	24.13 ± 2.66	0.305
	Control (n = 6)	1.78 ± 0.15		76.41 ± 15.33		31.31 ± 3.75	
Estrus	PNA (n = 5)	2.82 ± 0.33	0.030	63.57 ± 6.83	0.424	31.85 ± 3.06	0.177
	Control (n = 6)	1.56 ± 0.32		70.72 ± 21.96		25.57 ± 2.54	
Metestrus	PNA (n = 6)	2.73 ± 0.44	0.874	93.11 ± 28.48	0.242	30.79 ± 5.13	0.528
	Control (n = 5)	2.44 ± 0.34		45.12 ± 4.20		37.31 ± 2.78	
Diestrus	PNA (n = 6)	4.05 ± 1.09	0.305	60.17 ± 9.98	0.554	26.20 ± 3.78	0.474
	Control (n = 6)	2.29 ± 0.22		62.04 ± 8.20		22.49 ± 5.07	
Total	PNA (n = 23)	3.02 ± 0.32	0.003	79.07 ± 10.98	0.323	28.08 ± 1.91	0.764
	Control (n = 23)	2.00 ± 0.14		64.37 ± 7.29		28.82 ± 2.10	