J. steroid Biochem. Vol. 22, No. 5, pp. 631–634, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

PLASMA ANDROGENS DURING THE LUTEAL PHASE IN A CASE OF TRUE HERMAPHRODITISM WITH BILATERAL OVOTESTIS

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(*Received* 6 *April* 1984)

Summary—A true hermaphrodite with a bilateral ovotestis and a 46 XX karyotype was studied. This 14-year old subject developed ambiguous puberty with bilateral gynecomastia and stage IV pubic hair. Relatively high level of testosterone (T) (2.80 ng/ml), was found. The 5α-reductase activity for T in the pubic skin was similar to that observed in normal adult males. A hemorrhagic corpus luteum in the left ovotestis was observed at laparotomy. The luteal phase immediately after dexamethasone administration (1 mg/day for 7 days) was characterized by a significant decrease of plasma androgens, T and androstenedione (A). The constantly low level of T (0.30 ng/ml) during the luteal phase in this subject did not appear to be due to the previously administered dexamethasone. This decrease of T production in the luteal phase might be secondary either to the increase of the estradiol-17β (E_2) secreted by the corpus luteum or to the decrease of LH levels. Both mechanisms might act concomitantly.

INTRODUCTION

Ovulation has been reported to occur spontaneously in half of the patients with proved ovotestis [1, 2] and might turn out to be more frequent after surgical removal of the testicular portion of the gonad [3]. It can also be induced by administration of hMG followed by hCG [4]. Not much data concerning plasma androgen levels during the luteal phase in true hermaphroditism with a bilateral ovotestis is available [4, 5]. Therefore, we wish to present our data concerning the androgen pattern during the luteal phase in a patient with true hermaphroditism.

EXPERIMENTAL

Case report

A 14-year old subject was referred because of bilateral gynecomastia. He was 46 XX, H–Y antigen: 18 (H–Y antigen for normal males: 20 ± 3.10, for normal females: 9.3 ± 2.0) [6]. This subject who was brought up as a boy was operated at twelve because of a urogenital sinus opening into the perineum. Over the year before admission, the breasts developed (Tanner stage IV) and axillary and pubic hair appeared (stage IV).

Physical examination was negative except abnormal external genitalia. A small 6 cm phallus was present with marked chordee. Because of a urethroscrotal fistula 3 cm below the phallus the patient had to keep sitting for urinating. No palpable gonads were present in either the folds or the inguinal areas. The pelvic ultra-sonography confirmed the presence of a uterus. There was a vaginal opening into a urogenital sinus. Surgery was performed when the existence of a congenital adrenal hyperplasia had been ruled out. A bilateral ovotestis was found at laparoscopy with a ratio of ovarian mass to testicular mass of 4/1 on the right side and of 8/1 on the left. The ovarian portion of each ovotestis was adjacent to a Fallopian tube. A uterus with a cervix was present and the vagina was infantile. Hypoplastic Wolfian ducts were found adjacent to the ovotestis, (Fig. 1).

In the testicular portion of the ovotestis, Leydig cells were observed as well as seminiferous tubules with only Sertoli cells. Spermatogenesis could not be demonstrated. The ovarian portion was histologically normal with primary and preantral follicles on the right side and with a hemorrhagic corpus luteum on the left side. Hysterectomy with bilateral castration was performed.

Methods

Plasma FSH and LH were determined by a double antibody radioimmunoassay [7]. Results were expressed as nanograms per ml of the LER 907 reference preparations for FSH and LH. All plasma

The following trivial names and abbreviations have been used: Androstanediol: 5α-androstane-3α,17β-diol (3α-diol); Androstenedione: 4-androstene-3,17-dione (A); Cortisol: 11β,17,21-trihydroxy-4-pregnene-3, 20-dione (F); Dehydroepiandrosterone: 3β-hydroxy-5-androsten-17-one (DHA); Dehydroepiandrosterone sulfate (DHAS); Estradiol-17β: 1,3,5(10)-estratriene-3,17β-*diol* (E2); Estrone: 3-hydroxy-1,3,5(10)-estratrien-17-one (E1); 17-Hydroxyprogesterone: 17-hydroxy-4-pregnene-3,20-dione (17-OHP); Progesterone: 4-pregnene-3,20-dione (P); Testosterone: 17β-hydroxy-4-androsten-3-one (T).

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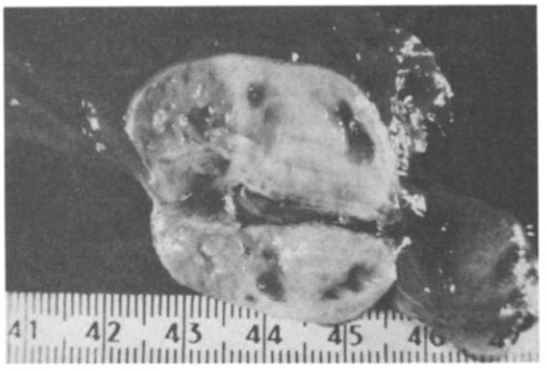

Fig. 1. Mascroscopic aspect of the ovotestis.

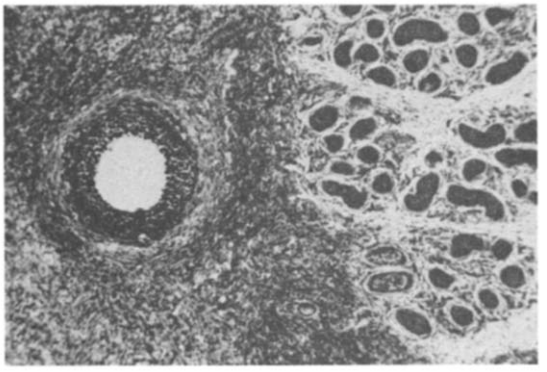

Fig. 2. Ovarian follicles adjacent to testicular tissue are shown. Seminiferous tubules contain only Sertoli cells.

steroid determinations were performed by radioimmunoassay after column chromatography on celite for testosterone (T) androstenedione (A) and dehydroepiandrosterone (DHA) [8] or Sephadex LH 20 for progesterone (P), 17-hydroxyprogesterone (17-OHP) [9] and estrone (E1), estradiol-17β (E2) [10] and cortisol (F) and 11-deoxycortisol (S) [11]. Testosterone-estradiol binding globulin capacity (TeBG) and apparent free testosterone concentration (AFTC) were determined according to the technique of Vermeulen *et al.*[12] and urinary 5α-androstane-3α,17β-diol glucuronide (3α-diol) according to that of Berthou *et al.*[13].

Study protocol

I—Determination of baseline plasma hormone levels was performed on blood samples drawn from an antecubital vein at 9 a.m. and immediately centrifuged. Plasma was then separated and kept frozen at $-20°C$ until assays were carried out. Urinary 3α-diol was evaluated when the patient was seen the first time (Table 1-1).

II—Adrenal stimulation was obtained by I.M. injection of 1.0 mg long-acting synthetic ACTH, 1–24 Synacthen® to rule out an adrenal enzymatic deficiency. Blood samples were collected at the basal state and 3 h after the injection [14]. One month after this test, dexamethasone was administered for 7 days at a daily dose of 1 mg. Blood samples were collected before and 1, 2, 3 and 7 days after dexamethasone administration. Hysterectomy with bilateral castration was performed the day after the last blood collection.

III—Testosterone supra-pubic skin metabolism was studied in this patient and in four normal adult men. One hundred milligrams of tissue was incubated with 2430 pmol of [4-^{14}C]T (CEA, France; 50 mCi/mM) in a total volume of 5 ml of 0.067 M phosphate buffer (pH 7.4) supplemented with NADPH (0.5 mM). Incubation was carried out at 37°C, stopped after 30 min by the addition of chilled acetone and then cooled to $-20°C$. Extraction was performed with ethyl acetate. Substrates and radiometabolites present in the crude extracts were separated by thin-layer chromatography on silica gel developed twice in benzene–ethanol (9:1, v/v). Radioactive metabolites were located by autoradiography and their R value compared with that of authentic standards run in parallel on the same plate [15].

RESULTS

ACTH test was followed by a decrease of T (2.80–0.90 ng/ml) and a normal increase of F (200–500 ng/ml), of its precursors (17-OHP: 1.10–1.70 ng/ml; S: 0.80–2.80 ng/ml) as well as of DHA (5.30–8.20 ng/ml). Thus an enzymatic defect in the

Table 1. Gonadotropin and steroid levels (ng/ml) in a case of true hermaphroditism

		Late follicular phase			Days after the end of dexamethasone administration Luteal phase					
		(1)	(2)[1]		+1	+2	+3	+7	+8	+14
T	(ng/ml)	2.80	3.00	Dexamethasone	0.26	0.35	0.37	0.30	Bilateral castration	0.05
A	(ng/ml)	2.00	3.00	one mg/day for	0.50	1.10	1.30	0.75	and hysterectomy	0.50
DHA	(ng/ml)	5.30	5.20	7 days	0.30	3.30	3.30	1.50		0.50
DHAS	(ng/ml)	840	450		135	430	540	500		440
P	(ng/ml)		0.70		8.00	5.50	4.00	0.50		0.05
17-OHP	(ng/ml)	1.70	4.50		1.20		2.50	1.0		0.50
F	(ng/ml)	200	220		10			120		
E1	(pg/ml)		86		80	80	60	25		10
E2	(pg/ml)	92	140		110	107	100	45		20
FSH	(ng/ml)	1.30			0.50	0.50	0.55	1.0		11.40
LH	(ng/ml)	2.40			0.20	0.25	0.20	0.60		3.20

[1]One month elapsed between the first and the second studies; days +1, +2, +3, +7 after dexamethasone treatment coincide with the luteal phase.

biosynthetic pathways of cortisol could be ruled out [14].

Plasma baseline levels of steroids and gonadotropins are reported on Table 1. Whilst T and AFTC concentrations were quite subnormal when compared with normal adult men (T: 3.40–9.00 ng/ml; AFTC: 5.00–20.00 ng/100 ml), those of E2 were rather high (normal males: 10–40 pg/ml) particularly the day before dexamethasone administration. In this sample 17-OHP level was found to be elevated also.

After dexamethasone administration for 7 days, the levels of P and E_2, retrospectively analysed, were consistent with the presence of a corpus luteum and in fact it was visualized at laparotomy. Thus the high E_2 and 17-OHP levels observed before dexamethasone treatment could be considered as indicative of a late follicular phase. All androgens and more particularly T and DHA decreased dramatically after dexamethasone and reached approx. 10% of their initial level. It is noteworthy that T levels remained low even 7 days after dexamethasone withdrawal whilst A, DHA and DHA sulfate increased throughout this period.

During this luteal phase the gonadotropin concentrations were low and began to increase the day of the operation.

Urinary 3α-diol glucuronide level, 160 μg/24 h, was within the normal adult men range (71–338 μg/24 h).

The study of T metabolism in the pubic skin showed that the amount of the 5α-reduced metabolites formed after incubation (300 pmol/30 min/100 mg) was also within the normal range for adult men (300–340 pmol/30 min/100 mg).

DISCUSSION

A bilateral ovotestis is observed in 25% of the cases of true hermaphroditism reported in the literature [1, 2]. In the patient described in this paper, ovarian mass was greater than the testicular one, the ratios being 4:1 and 8:1 (class II of the Jones classification). Both gonads were intra-abdominal and there were well-developed Mullerian ducts whereas the Wolfian ducts were rather atrophied. This 14-year old virilized subject displayed plasma T and AFTC levels higher than those observed in age-matched girls and rather comparable to what can be seen in post-pubertal boys. Similarly urinary 3α-diol glucuronide excretion was within the normal adult men range and this was also true for the cutaneous 5α-reduction of testosterone.

The occurrence of a spontaneous ovulation in such a virilized true hermaphrodite is quite unexpected since it has been suggested that androgen production excess might be the cause of anovulation in polycystic ovarian-like syndromes [16]. In this patient we have no data concerning the ovulatory period which occurred during dexamethasone administration, as no blood sample was collected during this period. However, the high 17-OHP and E_2 levels with the value of P which was higher than during follicular phase before dexamethasone administration could be consistent with the late follicular phase. In fact this was really the case since 7 days later P and E_2 levels suggested the existence of a functional corpus luteum which was evidenced later at laparatomy. Thus ovulation does not seem to have been induced by androgen suppression following dexamethasone administration. Moreover it occurred while the patient was receiving dexamethasone. This treatment did not appear to prevent the preovulatory gonadotropin surge and this is in keeping with Lachelin *et al.*[17] data who used equivalent doses as ours and even with those of Kim *et al.*[18] who used double doses. However glucocorticoid suppression of midcycle LH peak [19] and of LH response to LHRH [20, 21] or clomiphene [22] has been reported but this effect has been observed with far larger doses than those used by us, by Lachelin *et al.*[17] and by Kim *et al.*[18].

Dexamethasone administration induced a dramatic drop of all plasma androgens and more particularly DHA. After dexamethasone withdrawal DHA and its sulfate increased rapidly to reach almost pretreatment levels 3 days later. The increase of androstenedione was more gradual whereas T remained at very low levels. Since T was shown to originate predominantly from the testicular portion of the gonad [5] the persistence of its decreased concentration with concomitant A and DHA increasing levels might be explained by a direct action of E_2 on the Leydig cells as it has already been described *in vivo* [23] and *in vitro* [24]. Thus as the patient was in the luteal phase, it might be hypothesized that the increasing E_2 levels had induced an inhibition of T biogenesis in the testicular portion of the ovotestis. Alternatively, T decrease might also be secondary to modification of LH secretion during the luteal phase. Gonadotropin levels might not be sufficient to stimulate the steroidogenesis in the Leydig cells and indeed Aiman *et al.*[5] observed 11 days after unilateral removal of an ovotestis a concomitant increase of LH and T levels. Nevertheless, the two mechanisms discussed above might be concomitantly responsible for the low T production by the testicular portion of the ovotestis.

Acknowledgements—We are indebted to Dr F. Berthou (Lab. Pr Floch) for urinary androstanediol and to Dr J. Caroff for gonadotropin assays and to Dr P. Youinou for assistance in the preparation of the manuscript.

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