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Feminine Patterns of Androgen Metabolism and Presence of Δ^5,3β-Hydroxysteroid Oxidoreductase in Target Organs of Genetic Rat Male Pseudohermaphrodites (38143)

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Bardin *et al.*, have extensively studied an inherited form of male pseudohermaphroditism in the Stanley-Gumbreck rat (1). These pseudohermaphrodites are genetic males, have testes, but otherwise have a female phenotype. The defect is transmitted by the carrier females to half her male offspring. Although the pseudohermaphrodites have lowered testicular testosterone production associated with a selective deficiency in testicular 17-ketosteroid reductase (1–3), the primary genetic defect of this animal appears to be an inherited end-organ insensitivity to androgens reflected at the cellular level by deficient target organ androgen-binding proteins (1).

We have recently reported that the wolffian ducts and the genital tubercles of term rat male fetuses contain an active Δ^5,3β-hydroxysteroid oxidoreductase and 17-ketoreductase system so that they convert dehydroepiandrosterone to androstenedione, testosterone, and 5α-reduced products (4).

In this report we have determined whether the genital skin and preputial glands of rat male pseudohermaphrodites, their King-X Holtzman male littermates and females can metabolize dehydroepiandrosterone, androstenedione, and testosterone to 5α-reduced products; and whether these tissues of pseudohermaphrodites have a deficiency of 17-ketoreductase as do their testes. We have determined also whether the pattern of the metabolism of these steroids differs among males, females and pseudohermaphrodites.

Materials and Methods. Adult male pseudohermaphrodites and their King-X Holtzman male littermates and females of 6 to 7 mo of age were purchased from Stanley-Gumbreck (Oklahoma City, Okla.). The preputial skin (genital skin) and preputial glands were dissected, weighed, and used immediately after removal from the animal for assay of steroidogenesis as described below.

Substrates. Substrates used were dehydroepiandrosterone-7-^{3}H (10 mCi/mole), androstenedione-1,2-^{3}H (48 mCi/mole) and testosterone 1,2-^{3}H (51 mCi/mole). The commercially obtained steroids were obtained from New England Nuclear, Boston, Massachusetts. Unlabelled steroids were obtained from Sigma Chemical Company, St. Louis, Missouri, or Steraloids, Pawling, New York. The labelled steroids were found to be better than 98% radiochemically and chemically pure and the unlabelled steroids were found to have no contaminants by thin-layer and gas-liquid chromatography prior to use.

Tissue Preparation. A homogenate of either the prepuce (genital skin), or preputial gland, was prepared using a power-driven glass Teflon homogenizer at a tissue concentration of 100 mg/ml in a buffered medium. The homogenate was filtered through a double layer of nylon mesh. The medium consisted of 66 m*M* potassium phosphate buffer, pH 7.4.

Enzymatic Activity. The conversion of labelled dehydroepiandrosterone, androstenedione, or testosterone, as substrate into the tritiated products: 3β,17β-dihydroxy-5-an-

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TABLE I. Products Formed by Preputial Glands.

Substrate	Group	No.	Products ng Product/hr./10 mg tissue							
			Δ^5-diol****	3α-diol	T	DHA	DHT	AND	ADD	ASD
DHA-7-^{3}H	Male	10	102 ± 45	51 ± 18	—	570 ± 84	816 ± 198	228 ± 39	39 ± 39	1200 ± 168
	Pseudo	8	273 ± 61*	120 ± 21*	—	663 ± 54	1311 ± 174*	123 ± 36*	82 ± 22**	384 ± 84*
	Female	6	192 ± 38*	131 ± 9*	—	570 ± 30	1041 ± 156	144 ± 51*	82 ± 33**	288 ± 93*
ADD-1, 2-^{3}H	Male	11	—	324 ± 54	39 ± 36	—	843 ± 86	432 ± 171	249 ± 184	858 ± 288
	Pseudo	9	—	648 ± 120*	69 ± 15***	—	726 ± 99	645 ± 213	51 ± 9*	252 ± 87*
	Female	9	—	552 ± 24	75 ± 18**	—	528 ± 201*	570 ± 171	84 ± 84*	327 ± 135*
Test-1, 2-^{3}H	Male	10	—	414 ± 192	66 ± 36	—	1452 ± 159	180 ± 39	126 ± 27	273 ± 120
	Pseudo	8	—	819 ± 222*	78 ± 60	—	849 ± 165*	555 ± 153*	81 ± 21*	204 ± 129
	Female	8	—	771 ± 194*	120 ± 105	—	861 ± 450*	531 ± 177*	105 ± 39	147 ± 48

* $P < .001$ compared to males.

** $P < .01$ compared to males.

*** $P < .04$ compared to males.

**** Abbreviations: 3α-diol: 3α, 17β-dihydroxy-5α-androstane; Δ^5-diol: 3β,17β-dihydroxy-5-androstene; T: testosterone; DHA: 3β-hydroxy-5-androsten-17-one; DHT: 17β-hydroxy-5α-androstan-3-one; AND: 3α-hydroxy-5α-androstan-17-one; ADD: 4-androstene-3,17-dione; ASD: 5α-androstane-3,17-dione.

TABLE II. Products Formed by Genital Skin.

Substrate	Group	No.	Products ng Product/hr./10 mg tissue							
			Δ^5-diol ****	3αdiol	T	DHA	DHT	AND	ADD	ASD
DHA-7-^{3}H	Male	10	51 ± 36	—	—	2489 ± 78	—	24 ± 24	60 ± 45	60 ± 72
	Pseudo	6	141 ± 117 °	—	—	2316 ± 312	—	120 ± 90 *	84 ± 33	54 ± 21
	Female	9	234 ± 135 *	—	—	951 ± 390 *	—	456 ± 222 *	975 ± 306 *	—
ADD-1, 2-^{3}H	Male	10	—	21 ± 9	18 ± 12	—	33 ± 12	150 ± 45	2460 ± 72	168 ± 54
	Pseudo	9	—	60 ± 18 *	45 ± 30 **	—****	165 ± 102 *	912 ± 279 *	1407 ± 306 *	195 ± 72
	Female	10	—	42 ± 18	45 ± 27 **	—	93 ± 24 *	777 ± 273 *	1524 ± 516 *	144 ± 15
Test-1, 2-^{3}H	Male	10	—	222 ± 138	1926 ± 279	—	264 ± 162	51 ± 30	279 ± 114	45 ± 45
	Pseudo	6	—	1560 ± 279 *	474 ± 78 *	—	348 ± 186	225 ± 42 *	168 ± 33	54 ± 24
	Female	9	—	987 ± 84 *	1007 ± 120 *	—	261 ± 84	162 ± 39 *	285 ± 39	51 ± 9

* $P < .001$ compared to males.

** $P < .01$ compared to males.

*** $P < .04$ compared to males.

**** Abbreviations: See Table I.

drostene, 3α,17β-dihydroxy-5α-androstane, testosterone, 3β-hydroxy-5α-androstane-17-one, 3α-hydroxy-5α-androstane-17-one, 17β-hydroxy-5α-androstane-3-one, androstenedione, or 5α-androstane-3,17-dione by the genital skin or preputial gland was determined by a modification of our previously published micro method (4). 100 μl of homogenate containing one 1 μG (1,000,000 cpm.) substrate in 20 μl of dimethylsulfoxide (DMSO), 145 m*M* NAD, 18 m*M* NADP, 66 m*M* glucose-6-phosphate and 5 U. glucose-6-phosphate dehydrogenase were taken up in a total volume of 200 μl with phosphate buffer, pH 7.4. Incubation was for 20 min at 37°. The reaction was terminated with one ml ethanol/acetone (1/1:v/v). The labelled products were separated by a partition system on TLC, using propylene glycol as stationary phase, and carbon tetrachloride/cyclohexane (90/10:v/v) as mobile phase (Solvent I). Label peaks were also identified as trimethylsilylether derivatives by radio-gas-liquid chromatography using 3% OV-210, and by recrystallization to constant specific activity as previously described (4). A product was not considered identified unless it had fulfilled the criterion of no contamination by any other metabolite by the above methods.

The conditions of incubation were chosen as optimum after kinetic studies had established saturating amounts of substrate and that the production of products was proportional to the amount of homogenate, as well as to the time of incubation up to 1 hr.

Statistics. Significance was calculated according to the method of Student's *t*. ± = one standard deviation.

Results. DHA as substrate. The conversion of DHA-7-^{3}H by preputial glands is considerably greater than that by genital skin, but the same products are formed as those from androstenedione or testosterone with the exception of Δ^5-diol (Tables I and II). The preputial glands of both the pseudohermaphrodite and the female produce significantly more Δ^5-diol, 3α-diol, ADD, and DHT, but less AND and ASD than the littermate male (Table I). The genital skin of both the female and the pseudohermaphrodite produces significantly more 3α-diol and AND than that of the littermate male (Table II).

Androstenedione as substrate. The preputial glands of the pseudohermaphrodite and female produce significantly more 3α-diol, T, and AND but less DHT, ASD, and residual ADD than do those of the male (Table I). The genital skin of the pseudohermaphrodite and female produce significantly more 3α-diol, T, DHT and AND than do those of the littermate male (Table II).

Testosterone as substrate. Both the preputial glands and genital skin of the pseudohermaphrodite and female produce significantly more 3α-diol and androsterone than do those of the male (Tables I and II). The preputial glands of both the pseudohermaphrodite and female produce significantly less DHT than do those of the littermate males.

Enzyme Activities. These product differences are a reflection of the fact that the tissues of the female and pseudohermaphrodite have significantly increased 17-ketoreduction, 5α-reduction, and 3α-hydroxysteroid oxidoreduction in most cases (Table III).

Discussion. The present results indicate that the pattern of androgen metabolism in the genital skin and preputial glands of the rat male pseudohermaphrodite resembles that of the corresponding tissues of the female and is significantly different from that of its littermate male. The primary differences appear to be an elevated amount of 17β-hydroxylated and 3α-hydroxylated 5α-androstane products. The observation that these tissues in the adult animals have an active Δ^5,3β-hydroxysteroid oxidoreductase enzyme system confirms our previous observation of the presence of this enzyme system in the corresponding tissues of the term fetus (4).

The present finding of an increase of 17-ketoreductase in the target organs of the pseudohermaphrodite rat in addition to the observation of the presence of this enzyme in the adrenals of this animal (2) suggests that either the adrenals and target organs contain an isozyme of this enzyme or, as has been previously suggested (1–3, 5), that the testicular deficiency of this enzyme is not the primary genetic defect but secondary to the deficiency of androgen receptor proteins. Thus, the reduction in testosterone output by the testes associated with a deficiency of 17-ketoreductase is probably a result of the primary androgen unresponsive defect rather than a cause of it.

TABLE III. Combined Enzyme Activities in Preputial Glands and Genital Skin According to Substrate.

Preputial Gland	Enzyme Activity	Substrate	Activity ng Products/hr./10 mg tissue Male	Pseudo	Female
	17-Ketoreduction	DHA***	816 ± 64	1704 ± 66**	1365 ± 60**
		ADD	1206 ± 59	1443 ± 64**	1155 ± 64
	5α-Reduction	DHA	2295 ± 106	1938 ± 105	1605 ± 77
		ADD	2457 ± 200	2271 ± 173	1977 ± 133
		Test	2319 ± 128	2427 ± 167	2310 ± 217
	3α-Hydroxysteroid Oxidoreduction	DHA	279 ± 29	243 ± 29	276 ± 30
		ADD	656 ± 113	1293 ± 167**	1080 ± 98**
		Test	594 ± 116	1374 ± 188**	1302 ± 186**
Genital Skin					
	17-Ketoreduction	DHA	51 ± 36	234 ± 135**	141 ± 117*
		ADD	72 ± 11	270 ± 50**	180 ± 23**
	5α-Reduction	DHA	84 ± 48	456 ± 222**	174 ± 56**
		ADD	372 ± 30	1332 ± 118**	1656 ± 83**
		Test	582 ± 94	2187 ± 133**	1461 ± 54**
	3α-Hydroxysteroid Oxidoreduction	DHA	24 ± 24	120 ± 90**	456 ± 222**
		ADD	171 ± 27	972 ± 148**	819 ± 146**
		Test	273 ± 134	1785 ± 161**	1149 ± 62**

* $P < .04$ when compared to males.

** $P < .001$ when compared to males.

*** Abbreviations: See Table I.

Genital skin of adult females and of adult males feminized by in utero and postnatal treatment with cyproterone acetate, a progestin with strong antiandrogenic effects (6), convert less testosterone to 5α-reduced metabolites than that of intact adult males (7). The target organs, including wolffian ducts and genital tubercles of male fetuses, which have been feminized by in utero treatment with cyanoketone, a specific inhibitor of Δ^5, 3β-hydroxysteroid oxidoreductase, or cyproterone acetate, have female patterns of androgen metabolism present at birth (4). Therefore, it can be concluded that blockade of the masculinizing action of testosterone either by inhibiting its biosynthesis or by preventing target organ uptake within the fetus can feminize the pattern of androgen metabolizing enzymes in the genital skin and preputial glands at birth.

We have reported that the liver of the pseudohermaphrodite has a female character of steroid metabolism in that 5α-reduced metabolites are formed and excreted, rather than the hydroxylated metabolites characteristic of males (8–10). The mechanisms which control qualitatively and quantitatively different sex-dependent patterns of enzyme levels in the liver are irreversibly determined by the testis of the male rat in the first few days of life (11–13). Male rats castrated in the neonatal period develop after puberty a female type of hepatic metabolism. Testosterone replacement of the neonate irreversibly restores the male pattern, but given after puberty produces only a transient male pattern. We have produced the reversible type of testosterone induced masculinization of liver patterns of steroid metabolizing enzymes by large doses of testosterone administered to the adult pseudohermaphrodite (10).

Thus, it appears that there is a sex-dependent difference in the pattern of androgen metabolizing enzymes which may be determined in the genital skin and preputial glands at or before birth. The failure of the XY rat male pseudohermaphrodite to attain a masculine pattern of androgen metabolism in these target organs can be attributed to the fact that the inborn feminine program is allowed to be expressed by virtue of the genetic defect of testosterone unresponsiveness in this animal in utero due to a deficiency of androgen receptors.

Summary. The metabolism of dehydroepiandrosterone-7-^{3}H, androstenedione-1,2-^{3}H, and testosterone-1,2-^{3}H by the preputial glands and genital skin (prepuce) was determined in genetic male pseudohermaphrodites, and in their littermate King-X Holtzman males and females. Both tissues in each of the three animals have an active Δ^5-3β-hydroxysteroid oxidoreductase system capable of transforming dehydroepiandrosterone to testosterone and testosterone metabolites in a similar fashion to that of the woffian duct and genital tubercle of the fetus of this species as previously reported. The present results also indicate that the pattern of androgen metabolism in both tissues of the rat male pseudohermaphrodites has an elevated amount of 17β-hydroxylated and 3α-hydroxylated 5α-androstane products resembling that of the corresponding tissues of the female and is significantly different from that of its littermate male. Thus, it appears that there is a sex dependent difference in the pattern of androgen metabolizing enzymes and target organs which may be determined at or before birth. The failure to attain a masculine pattern in the male pseudohermaphrodite can be attributed to this animal's inherited deficiency of androgen receptor proteins.

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