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COMPARISON OF ANDROGEN SYNTHESIS IN HUMAN FETAL TESTIS AND ADRENAL: 3β -HYDROXYSTEROID DEHYDROGENASE-ISOMERASE AND 17β -STEROID DEHYDROGENASE ACTIVITIES

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SUMMARY

To assess comparative 3β -hydroxysteroid dehydrogenase-isomerase and 17β -steroid dehydrogenase activities in human fetal testis and adrenal, equal weights of adrenal and testicular tissue from the same fetuses were incubated with dehydroepiandrosterone or pregnenolone, under identical conditions. Tissues from five fetuses were investigated. The following products were isolated and quantitated: dehydroepiandrosterone, androstenediol, androstenedione and testosterone. The 3β -hydroxysteroid dehydrogenase-isomerase activity, expressed as pmoles found in androstenedione plus testosterone per mg protein incubated, was 3.5-138 times higher in testicular than in adrenal tissue. 17β -Steroid dehydrogenase activity expressed as pmoles found in testosterone plus androstenediol per mg protein incubated was 73-313 in testicular tissue compared to 0-2.64 in adrenal tissue. When the same activities were expressed in terms of activity per total adrenal and testicular tissue per fetus, the 17β -steroid dehydrogenase activity was still considerably higher in testes than in adrenals, while the 3β -hydroxysteroid dehydrogenase-isomerase activity was higher in the testes of the three smaller fetuses investigated. These data provide additional evidence that the human fetal testis is geared to the formation of testosterone which is essential for genital development in the male fetus.

INTRODUCTION

We have recently compared the capacity of fetal adrenals and testes, obtained from the same human fetuses, to sulfurylate pregnenolone, dehydroepiandrosterone and testosterone¹. Although these tissues are of similar embryonic origin, marked differences in their capacity to synthesize the sulfate esters of these three steroids were observed. When equal weights of adrenal and testicular tissue were incubated

The following abbreviations and trivial names are used: pregnenolone, 3β -hydroxy-5-pregnen-20-one; 17β -hydroxypregnenolone, 5-pregnen- $3\beta,17$ -diol-20-one; dehydroepiandrosterone, 3β -hydroxy-5-androsten-17-one; androstenediol, 5-androstene- $3\beta,17\beta$ -diol.

with equimolar amounts of steroid substrate, considerably less sulfurylation of pregnenolone and dehydroepiandrosterone occurred in the testes. No formation of testosterone sulfate occurred in the human fetal testes while 37 and 77% of the testosterone incubated was found as the sulfate ester in the adrenals of the same fetuses. This finding is consistent with the hypothesis that testosterone is kept in an active form in the testis and supports the concept that free testosterone, or a free steroid product of testosterone, is necessary for genital development in the human male fetus.

To determine whether the free C₁₉-steroid products formed from pregnenolone and dehydroepiandrosterone in the testes and adrenals from these same fetuses also show significant differences, we isolated and quantitated dehydroepiandrosterone, androstenediol, androstenedione and testosterone from these tissues.

MATERIALS AND METHODS

Materials

[7-³H]Pregnenolone, spec. act. = 25.0 Ci/mmole and [7-³H]dehydroepiandrosterone, spec. act. = 25.1 Ci/mmole from New England Nuclear Co., were each purified in two paper chromatographic systems. An aliquot of each was mixed with the appropriate authentic crystalline steroid and recrystallized. The specific activity of the initial crystals was within 2% of the starting material. [¹⁴C]Androstenediol was prepared as described previously². Cofactors were purchased from Sigma Chemical Co.

Crown-rump and crown-heel lengths, weights, and estimated gestational age of each fetus and the total weights of the adrenals and testes from each fetus *plus* the amount of tissue used in each incubation are presented in Table I.

Incubation procedure

Approximately equal weights of adrenal and testicular tissue were homogenized, within 30 min of obtaining each fetus at hysterotomy, in Potter-Elvehjem all glass homogenizers in 1.0 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5 μmoles of each of the following cofactors: ATP, MgCl₂, and K₂SO₄. The homogenates

TABLE I

FETAL DATA AND AMOUNT OF TISSUE INCUBATED

Expt. No.	Substrate	Fetal data			Estimated gestational age (weeks)	Total weight of tissue (mg)		Weight of tissue incubated (mg)	
		Weight (g)	Crown-rump length (cm)	Crown-heel length (cm)		Adrenal	Testis	Adrenal	Testis
	Dehydroepiandrosterone	72	11	15	14*	299	36.5	27.5	28.5
	Dehydroepiandrosterone	127	13	19	16*	335	36.5	28.0	27.8
	Dehydroepiandrosterone	286	16	24	18*	1202	79	25.5***	62.9
	Pregnenolone	159	14	19	17*	593	61.4	68.5	70.7***
	Pregnenolone	480	18	30	21**	1882	123.6	41.5	46.3
								110.4	93.2

* Based upon crown-rump length³.

** Based upon stated last menstrual period.

*** Amount incubated without added cofactors.

were transferred to incubation flasks containing either 5400 pmoles of [^3H]dehydroepiandrosterone or [^3H]pregnenolone (8.2 μCi). Incubations were performed in a Dubnoff metabolic incubator for 3 h using $\text{O}_2\text{-CO}_2$ (95:5, v/v) as the gas phase. In addition, equal weight aliquots of adrenal tissue from two of the fetuses were homogenized in 0.05 M Tris-HCl buffer (pH 7.4), without added cofactors, and incubated with [^3H]dehydroepiandrosterone as described above. (These conditions were used in order to minimize sulfurylation of the [^3H]dehydroepiandrosterone.) Incubations were terminated by the addition of ethanol to a final concentration of 80%.

Tissue-less control incubations with each substrate were also performed.

Extraction and purification

Three extractions with 80% ethanol, followed by two with absolute ethanol were carried out for each tissue incubation. This was followed by a dichloromethane-water partition performed in a countercurrent fashion using six lower phase transfers. The following ^{14}C -labeled steroids were added to the dichloromethane fraction prior to chromatographic separation for quantitation of free steroid products: dehydroepiandrosterone ($28 \cdot 10^3$ dpm), androstenediol ($29 \cdot 10^3$ dpm), testosterone ($37 \cdot 10^3$ dpm) and androstenedione ($35 \cdot 10^3$ dpm).

The dichloromethane fraction from each incubation was submitted to paper chromatography in System 1 (Table II). The resulting peaks were submitted to additional paper chromatographic systems as follows: androstenediol and 17-hydroxypregnenolone, Systems 2 and 4; 17-hydroxypregnenolone acetate, System 5; testosterone, System 2; dehydroepiandrosterone and androstenedione, System 3. Final proof of identity was accomplished by recrystallization to constant specific activity of representative samples with authentic crystalline steroids.

Protein was determined by the method of Lowry *et al.*⁴.

RESULTS

Between 93 and 100% of the incubated radioactivity was recovered in the various incubations.

Table III depicts the amount of radioactivity found in the aqueous and organic fraction *plus* the amounts of free steroid products isolated and identified from the organic fraction in each incubation. The amount of fetal tissue utilized for each incubation expressed as mg of protein also is given.

3 β -Hydroxysteroid dehydrogenase-isomerase activity

This activity, expressed as pmoles of androstenedione *plus* testosterone per mg protein is presented in Table IV. Fetal testicular tissue from the same fetus exhibited from 3.5 to 138 times the activity found in adrenal tissue incubated under identical

TABLE II

PAPER CHROMATOGRAPHIC SYSTEMS EMPLOYED

No.	Solvent	by vol.
1	Heptane-benzene-methanol-water	66:34:80:20
2	Cyclohexane-benzene, formamide	1:1
3	Heptane-methanol-water	5:4:1
4	Toluene, propylene glycol	
5	Ligroine, propylene glycol	

TABLE III

FREE STEROIDS ISOLATED FOLLOWING INCUBATION OF FETAL ADRENALS AND TESTES WITH DEHYDROEPIANDROSTERONE OR PREGNENOLONE

Expt No.	Tissue	Protein* (mg)	Aqueous fraction (dpm × 10 ⁻³)	Organic fraction (dpm × 10 ⁻³)	Free steroid products isolated (dpm × 10 ⁻³)				
					17 Hy-droxy-pregne-nolone**	Dehydro-epiandro-sterone	Andro-steronediol	Andro-steronediene	Testo-sterone
<i>Dehydroepiandrosterone</i>									
1	Adrenals	1.60	17870	394	—	172	0	90	0
	Testes	1.54	7080	10709	—	5620	422	1466	1023
2	Adrenals	1.84	17380	392	—	121	0	14.3	0
	Adrenals***	1.68	578	17680	—	17030	4.2	128	0
3	Testes	1.94	6420	10900	—	7480	169	1760	305
	Adrenals	4.43	4480	13300	—	9460	13	580	8.2
	Adrenals***	4.57	979	15540	—	13160	17	537	0
	Testes	3.99	1630	16460	—	10870	388	1420	486
<i>Pregnenolone</i>									
4	Adrenals	3.00	16473	643	21	7.4	0	5.2	5.7
	Testes	2.60	2457	13928	192	4901	332	988	1307
5	Adrenals	6.54	9096	7980	1240§	2235	0	152	58
	Testes	6.03	1800	14040	318	3220	1520	239	2310

* Amount incubated.

** Amount of 17-hydroxypregnenolone was not corrected for losses

*** No cofactors added to incubation medium.

§ Lost a portion of this pool accidentally.

conditions. To establish whether the difference in the conversion of dehydroepiandrosterone to androstenedione in these two fetal tissues might be due to the much greater degree of sulfurylation observed in the adrenals compared to the testes (Table III aqueous fraction and Ref. 1), equal weight portions of adrenal tissue from two of the fetuses were incubated without added cofactors. This essentially eliminated sulfurylation (Table III). Under these conditions, the conversion of dehydroepiandrosterone to androstenedione was increased in the adrenal tissue from fetus No. 1, but no difference was observed in the adrenal tissue from fetus No. 2. The marked difference in the 3 β -hydroxysteroid dehydrogenase-isomerase activity in the two fetal tissues was still observed (Tables III and IV).

17 β -Steroid dehydrogenase activity

This activity, expressed as pmoles of androstenediol *plus* testosterone per mg protein, is presented in Table IV. In the adrenals, 17 β -steroid dehydrogenase activity was observed only in tissue from the three largest fetuses.

The range of activity found in all of the adrenal incubations varied from 0 to 2.64 pmoles per mg protein. In contrast, testicular tissue from the same fetuses exhibited 17 β -steroid dehydrogenase activity between 73 and 313 pmoles per mg protein.

DISCUSSION

The present study demonstrating markedly higher activities of the 3 β -hydroxysteroid dehydrogenase and 17 β -steroid dehydrogenase in testes compared to adrenals

TABLE IV

FETAL TESTICULAR AND ADRENAL 3β -HYDROXYSTEROID DEHYDROGENASE-ISOMERASE AND 17β -STERIOD DEHYDROGENASE ACTIVITIES

Fetus	Tissue	3β -Hydroxysteroid dehydrogenase-isomerase* (pmoles/mg protein)	17β -Steroid dehydrogenase** (pmoles/mg protein)
<i>Dehydroepiandrosterone</i>			
1	Adrenals	17.0	0
	Testes	485.0	313.0†
2	Adrenals	2.31	0
	Adrenals***	23.0	0.75
	Testes	320.0	73.0
3	Adrenals	40.0	1.44
	Adrenals***	35.0	0.69
	Testes	143.0	73.0 ††
<i>Pregnenolone</i>			
4	Adrenals	9.6	2.64
	Testes	127.0	191.0
5	Adrenals	0.51	0.57
	Testes	251.0	183.0

* 3β -Hydroxysteroid dehydrogenase-isomerase = testosterone plus androstenedione.** 17β -steroid dehydrogenase = androstenediol plus testosterone.

*** No cofactors added to incubation medium.

† Includes 1.5 pmoles per mg protein found in androstenediol-3-sulfate¹.†† Includes 31.5 pmoles per mg protein found in androstenediol-3-sulfate¹.

from the same fetus, when measured per mg of tissue protein, provides additional evidence for the marked differences in enzymic activities observed in these two tissues during the second trimester of pregnancy¹. The possibility that the observed differences of these activities are due to differences in the availability of pyridine nucleotide cofactors in the fetal adrenal and testis cannot be excluded.

Although the 3β -hydroxysteroid dehydrogenase-isomerase activity* was considerably lower in the fetal adrenal compared to the testis, this activity was observed in all of the adrenal incubations. The presence of this enzyme activity in incubation studies performed with human fetal adrenal tissue has been reported previously by several investigators⁵⁻⁷, and recently examined in this laboratory⁸.

Since adrenals are considerably larger than testes from the same fetus, and since this may influence the total amount of a given steroid synthesized by each tissue *in vivo*, the 3β -hydroxysteroid dehydrogenase-isomerase and 17β -steroid dehydrogenase activities are presented as pmoles per total tissue in Table V. Thus, if the total amount of tissue is taken into consideration, the 17β -steroid dehydrogenase activity is still markedly higher in testicular tissue compared to adrenal tissue in the five fetuses investigated. The 3β -hydroxysteroid dehydrogenase-isomerase activity is higher in testes from the three smaller fetuses (14-17 weeks gestational age), while in the two larger fetuses (18 and 21 weeks gestational age) this activity is higher in the adrenals, based upon total weight.

It is of interest to note that androstenediol, a possible intermediate in a biosynthetic pathway to testosterone which could exclude androstenedione as an inter-

* The 3β -hydroxysteroid dehydrogenase-isomerase activity with pregnenolone as substrate does not include radioactivity that might be associated with progesterone and 17-hydroxyprogesterone.

TABLE V

TOTAL 3β -HYDROXYSTEROID DEHYDROGENASE-ISOMERASE AND 17β -STERIOD DEHYDROGENASE ACTIVITIES

Expt No.	Fetus weight	Tissue	3β -Hydroxysteroid dehydrogenase activity (μ moles per total tissue)	17β -Steroid dehydrogenase activity (μ moles per total tissue)
<i>Dehydroepiandrosterone</i>				
1	72	Adrenals	216	0
		Testes	747	437
2	127	Adrenals	39	0
		Adrenals*	347	11
		Testes	620	142
3	286	Adrenals	2647	95
		Adrenals*	2417	77
		Testes	572	262
<i>Pregnenolone</i>				
4	159	Adrenals	32	17
		Testes	689	689
5	480	Adrenals	915	231
		Testes	765	1149

* No cofactors added to incubation medium.

mediate, was found in all testicular incubations irrespective of substrate. Rosner and Macome⁹ have reported the isolation of androstenediol as a product of both pregnenolone and dehydroepiandrosterone in incubations of adult human testicular homogenates. The secretion of large amounts of androstenediol by the adult human testis was recently reported by Laatikainen *et al.*¹⁰

The data presented in this study, *plus* our earlier observations that fetal testes do not sulfurylate testosterone¹, are in agreement with other studies demonstrating that the human fetal testis in early gestation is geared to the formation of the biologically active hormone, testosterone, which is essential for the development of external genitalia in human male fetuses¹¹.

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REFERENCES

- 1 R. B. Jaffe and A. H. Payne, *J. Clin. Endocrinol.*, 33 (1971) 592.
- 2 A. H. Payne and R. B. Jaffe, *J. Clin. Endocrinol.*, 33 (1971) 582.
- 3 B. M. Patten, *Human Embryology*, The Blakiston Co., New York, 1953, p. 185.
- 4 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
- 5 J. Ev. Jirasek, J. Sukova, A. Capkova, S. Rohling and L. Starka, *Endokrinologie*, 54 (1969) 173.
- 6 A. S. Goldman, W. C. Yakovac and A. M. Bongiovanni, *J. Clin. Endocrinol.*, 26 (1966) 14.
- 7 M. Niemi and A. H. Baillie, *Acta Endocrinol.*, 48 (1965) 423.
- 8 G. B. Serra, G. Pérez-Palacios and R. B. Jaffe, *Biochim. Biophys. Acta*, 244 (1971) 186.
- 9 J. M. Rosner and J. C. Macome, *Steroids*, 15 (1970) 181.
- 10 T. Laatikainen, E. A. Laitinen and R. Vihko, *J. Clin. Endocrinol.*, 32 (1971) 59.
- 11 A. Jost, *Recent Progr. Hormone Res.*, 8 (1953) 379.