PRESENCE OF TESTOSTERONE AND OTHER NEUTRAL STEROIDS IN HUMAN FETAL TESTES

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Free and sulfate-conjugated neutral steroids were isolated from the pooled testes of 33 human fetuses of 8.5 - 20.0 cm crown-rump length and the compounds were identified by gas-liquid chromatography and gas chromatography - mass spectrometry. Unconjugated pregnenolone and testosterone were the main compounds present. Thus it has been established that under *in vivo* conditions the fetal testis synthesizes androgens and that apparently the pathway pregnenolone → 17α-hydroxypregnenolone → dehydroepiandrosterone → androstenedione/5-androstene-3β,17β-diol → testosterone is preferred.

It is generally assumed that the differentiation of the male genitalia is dependent on induction by products of fetal testes (see *e.g.* 1). At the time of differentiation, the morphologic appearance of the interstitial cells of the fetal testes suggests marked activity (2) and *in vitro* studies have demonstrated synthesis of several steroid hormones, including testosterone, in human fetal testis tissue (3-6). An attempt to demonstrate the presence of C_{19}-steroids in fetal bovine testes was unsuccessful (7). Quite recently, testosterone, androstenedione, estrone and estradiol-17β were identified in the testes of fetal sheep (8). It seems that in man the endogenous steroids in fetal testes have not been analyzed.

This communication describes the identification and measurement of endogenous unconjugated neutral steroids and their mono- and disulfates in fetal testes of early and midpregnancy.

MATERIALS AND METHODS

Foetal testes were obtained during interruption of 33 pregnancies (of 12-24

Trivial and systematic names: Androstenedione: 4-androstene-3,17-dione, testosterone: 17β-hydroxy-4-androsten-3-one, dehydroepiandrosterone: 3β-hydroxy-5-androsten-17-one, progesterone: 4-pregnene-3,20-dione, pregnenolone: 3β-hydroxy-5-pregnen-20-one, 17α-hydroxypregnenolone: 3β,17α-dihydroxy-5-pregnen-20-one, estrone: 3-hydroxyestra-1,3,5 (10)-trien-17-one, estradiol-17β: estra-1,3,5 (10)-triene-3,17β-diol.

Table 1. Relative retention times (5α-cholestane = 1.00) of the neutral steroids identified in human fetal testes. Chromatographic conditions: 3 % QF-1, 215°; 2.2 % SE-30, 225°.

Identification	QF-1		SE-30	
	Compound from testes	Reference compound	Compound from testes	Reference compound
Unconjugated:				
Testosterone (MO-TMS) [x]	1.00 0.96	1.00 0.95	0.69	0.70
Androstenedione (MO)	1.07 1.11	1.06 1.10	0.65	0.66
Pregnenolone (MO-TMS)	1.25	1.27	1.03	1.00
Monosulfates:				
Dehydroepiandrosterone (TMS)	1.30	1.30	0.47	0.47
3β,16α-Dihydroxy-5-androsten-17-one (TMS)	1.39	1.43	0.85	0.85
Pregnenolone (TMS)	1.81	1.81	0.78	0.78
5-Pregnene-3β,20α-diol (TMS)	1.06	1.07	1.13	1.14
Disulfates:				
5-Androstene-3β,17α-diol (TMS)	0.47	0.48	0.54	0.53
5-Androstene-3β,17β-diol (TMS)	0.56	0.57	0.62	0.61

[x] Derivatives: MO-TMS=O-methyl oxime-trimethyl silyl; MO = O-methyl oxime; TMS = trimethyl silyl.

weeks duration) for socio-medical reasons. The fetus was delivered by abdominal hysterotomy and the testes were immediately dissected out, weighed, dropped in acetone/ethanol 1 : 1 and stored at -20°C until analyzed. The details of the analytical procedure used previously for the analysis of neutral steroid sulfates in human fetal adrenal and liver tissue have been published (9). In short, the method used in this study was the following: 1) Homogenization of the testes (2.24 g) in acetone/ethanol. 2) Chromatography of the extract on Sephadex LH-20. Fractions of unconjugated, mono- and disulfates of neutral steroids were obtained. 3) Solvolysis of steroid sulfates. 4) Fractionation of the free steroids on silicic acid. 5) Formation of trimethyl silyl, O-methyl oxime or O-methyl oxime-trimethyl silyl derivatives of the steroids. 6) Gas - liquid chromatography (GLC) on QF-1 and SE-30 liquid phases. 7) Gas chromatography--mass spectrometry (GC-MS) on an LKB-9000 instrument. A compound was considered to be identified when its GLC behavior on the two liquid phases used and its mass spectrum were the same as those of the reference compound. The concentration limit for a steroid to be identified according to these criteria was about 1 μg/100 g tissue wet weight. In quantitative analyses, a known amount of stigmasterol was added to the samples prior to GLC and the measurements were performed as described previously (10).

RESULTS

The amount of blood present in the testis samples analyzed was negligible, as shown by photometric analyses of the extracts. Therefore the steroids found were endogenous compounds of the testis tissue.

Testosterone, androstenedione and pregnenolone were identified in the fraction of unconjugated steroids (Table 1). Fig. 1 shows a GLC analysis of the silicic acid fraction containing testosterone and in Fig. 2 the mass spectrum of the O-methyl oxime-trimethyl silyl ether derivative of testosterone is seen. The mass spectrum was identical with that of the corresponding derivative of the reference testosterone. The bulk of the steroids measured were in the fraction of unconjugated steroids (Table 2).

In the monosulfate fraction, pregnenolone was present, too (Table 1). All the steroids in this fraction as well as in the disulfate fraction, had a 3β-hydroxy-Δ^5 structure. The main compounds in the fractions of sulfate-conjugated steroids were 16α-hydroxydehydroepiandrosterone and dehydroepiandrosterone (Table 2). 5-Androstene-3β, 17α-diol and 5-androstene-3β, 17β-diol were found only in the disulfate fraction.

DISCUSSION

In this study, endogenous unconjugated neutral steroids, and also their mono- and disulfates were identified and measured in pooled testes of 33

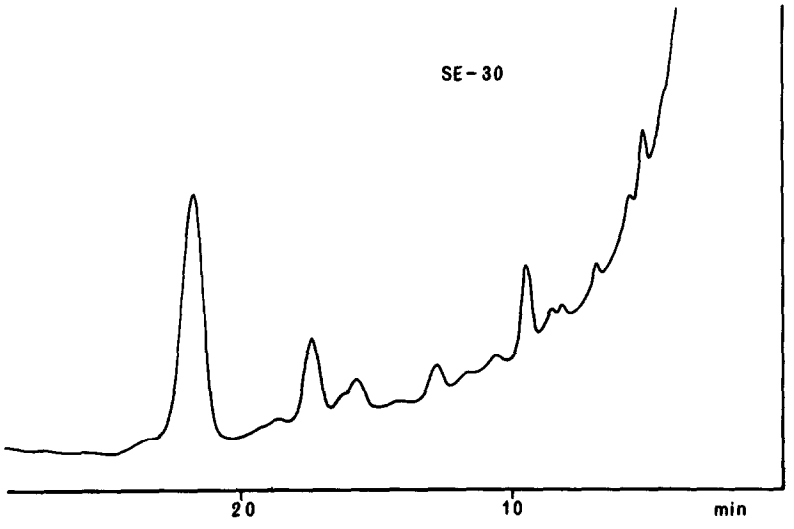

Fig. 1. A GLC analysis of a fraction (eluted with 33 % ethyl acetate in benzene from a 3 - g column of silicic acid) of the unconjugated neutral steroids in fetal testes. 2.2 % SE-30, 225°. The peak eluted at about 22 min represents the MO-TMS derivative of testosterone.

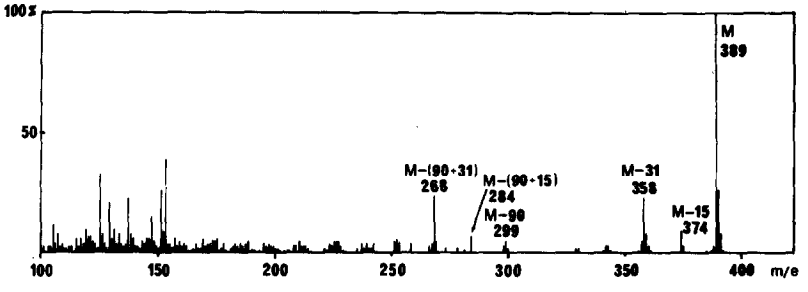

Fig. 2. Mass spectrum of the MO-TMS derivative of testosterone isolated from fetal testes.

fetuses of 12-24 weeks gestational age. The results demonstrate that there is an extensive metabolism of neutral steroids in vivo in fetal testicular tissue.

It is very interesting to observe that one of the main compounds

Table 2. Concentrations of neutral steroids in human fetal testes. The values are expressed as μg of the free steroid/100 g tissue wet weight.

Compound	Concentration
Unconjugated:	
Testosterone	170
Androstenedione	18
Pregnenolone	180
Monosulfates:	
Dehydroepiandrosterone	45
3β,16α-Dihydroxy-5-androsten-17-one	66
Pregnenolone	24
5-Pregnene-3β,20α-diol	22
Disulfates:	
5-Androstene-3β,17α-diol	2.6
5-Androstene-3β,17β-diol	1.6

identified was an active androgen, testosterone. There is considerable evidence that the differentiation of the male genitalia is dependent upon induction by products of the fetal testes (see 1). It is possible, therefore, that testosterone, shown now to be present in considerable amounts in human fetal testes at the time of the differentiation, might be involved in the induction process.

Another compound with a 3-keto-Δ^4 structure, androstenedione, a known precursor of testosterone, was identified. All the other compounds identified and measured had a 3β-hydroxy-Δ^5 structure. Although progesterone was searched for, it was not found to be present in fetal testes. Therefore, the pattern of neutral steroids in fetal testes (Tables 1 and 2) suggests that testosterone is synthesized through the pathway pregnenolone → 17α-hydroxypregnenolone → dehydroepiandrosterone → androstenedione/5-androstene-3β,17β-diol → testosterone. In vitro studies have shown that this pathway is operative in human fetal testes (4, 11). One step in this pathway, 17α-hydroxypregnenolone, was not found in testis tissue and it is possible that the side-chain-splitting activity of the tissue studied is so high that the compound does not accumulate in amounts detectable by the present method.

The presence of 16α-hydroxylating activity in the testes, previously shown to be present in *in vitro* studies (4), was demonstrated by the identification of 3β,16α-dihydroxy-5-androsten-17-one in the monosulfate fraction. Further, the reduction of a 17-keto group to 17α- and 17β-hydroxyl

and of a 20-keto group to 20α-hydroxyl seems to take place in fetal testis tissue *in vivo*, as has been shown in *in vitro* studies (4).

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