STEROID METABOLISM IN TESTIS TISSUE. CONCENTRATIONS OF TESTOSTERONE, PREGNENOLONE AND 5α-ANDROST-16-EN-3β-ol IN NORMAL AND CRYPTORCHID RAT TESTIS, AND IN ISOLATED INTERSTITIAL AND TUBULAR TISSUE

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1. Introduction

In the testicular metabolism of steroids, two areas have recently received great attention. One involves studies on the possible role of steroid sulphates in the biosynthesis of testosterone [1–4]; the other concerns the regulation of spermatogenesis by testicular steroids [5]. In order to obtain more information on these subjects in the rat, endogenous neutral steroids were identified and quantitated in pooled samples of rat testis, in isolated interstitial tissue and seminiferous tubules and in cryptorchid testes. Determinations were performed with gas–liquid chromatography and gas chromatography–mass spectrometry. The level of detection of a steroid was about 0.1 μg/100 g tissue wet weight. Testosterone (17β-hydroxy-4-androsten-3-one) was the main steroid found and, in addition, pregnenolone (3β-hydroxy-5-pregnen-20-one) and 5α-androst-16-en-3β-ol were identified. The concentration of testosterone was greatest in the sample of interstitial tissue. No sulphate-conjugated steroids were detected.

2. Experimental

Six pools (30.2–100.0 g) of testes from adult Sprague-Dawley rats were collected totalling 245.6 g (see table 2). The seminiferous tubules were collected according to the technique of Christensen and Mason [6]. Teased tubular pieces were immediately placed into acetone/ethanol (1:1). The final tissue residue, comprised of the interstitial cells and some blood vessels, was placed into another extraction vessel.

In order to exclude steroid contamination from the blood, the testes were infused with isotonic saline solution via the aorta.

Cryptorchidism was induced by closing the inguinal canal through a scrotal incision. The animals were killed 4 to 5 weeks after the operation, and their atrophic testes were treated similarly to the controls.

Samples were homogenized with an Ultra-Turrax homogenizer and ultrasonicator and then filtered. The residue was reextracted in 5 vol of chloroform/methanol (1:1) and the combined filtrates were chromatographed on a 20 g column of Sephadex LH-20 in chloroform/methanol (1:1) containing sodium chloride (0.01 mole/ℓ). Fractions of unconjugated, monosulphated and disulphated steroids were collected [7]. The fractions containing the sulphate conjugates were subjected to solvolysis and purified as described earlier [8]. The unconjugated steroid fraction was subjected to chromatography on a 5 g column of silicic acid eluted with 12% ethyl acetate in toluene and then stripped with methanol. The nonpolar fraction (16-unsaturated C_{19} steroids) was further purified on 2.5 g of hydroxyalkoxypropyl Sephadex (prepared as described in [9]) in 75% methanol in water. The polar fraction was similarly subjected to chromatography on hydroxyalkoxypropyl Sephadex using petroleum ether/chloroform (95:5) as solvent.

The steroids were converted to trimethylsilyl (TMS) and *O*-methyl-oxime-trimethylsilyl (MO-TMS) ether derivatives and were analysed by gas–liquid chromatography [8]. Gas chromatography-mass spectrometry was performed using a computerized

Table 1

Relative retention times of TMS and MO-TMS derivatives of steroids of rat testis tissue and those of corresponding derivatives of reference compounds. Conditions: 2.2% SE-30, 210° and 3% QF-1, 215°. Cholestane = 1.00.

Identification	SE-30				QF-1			
	Compound from the samples		Reference compound		Compound from the samples		Reference compound	
	TMS	MO-TMS	TMS	MO-TMS	TMS	MO-TMS	TMS	MO-TMS
Testosterone	0.63	0.71	0.63	0.70	2.70	0.98; 1.02	2.73	0.97; 1.02
Pregnenolone	0.78	0.99	0.78	1.00	1.84	1.01	1.84	1.02
5α-Androst-16-en-3β-ol*	0.164	–	0.164	–	0.236	–	0.236	–

* Conditions for the analysis of the TMS derivatives of 5α-androst-16-en-3β-ol: SE-30, 180° and QF-1, 185°

(Varian, Spectro System ™100 MS) combination instrument (Varian, Model CH-7). The energy of the bombarding electrons was 70 eV and the ionizing current 300 μA.

3. Results

Three steroids were identified; all of them in the unconjugated steroid fraction (table 1). The retention times and mass spectra of the derivatives of two of the compounds were identical with those of the corresponding derivatives of testosterone and pregnenolone, respectively (for mass spectra, see [10–12]). The third compound as the TMS ether derivative displayed retention times and a mass spectrum identical with those of the corresponding derivative of 5α-androst-16-en-3β-ol (table 1, fig. 1).

In all tissues examined testosterone had the highest concentration of the steroids identified and its concentration in the interstitial tissue was about 5-fold that in total testes (table 2). 5α-Androst-16-en-3β-ol was present in higher concentrations than pregnenolone in all the samples.

4. Discussion

According to the results of this investigation, the pattern of endogenous neutral steroids in rat testis is very different from that found in human [4] and boar [13]. The testes of these latter two species contain a large number of steroids many of which are present in high concentrations. In the rat, only testosterone, pregnenolone, and 5α-androst-16-en-3β-ol were found and no sulphate conjugates were detected,

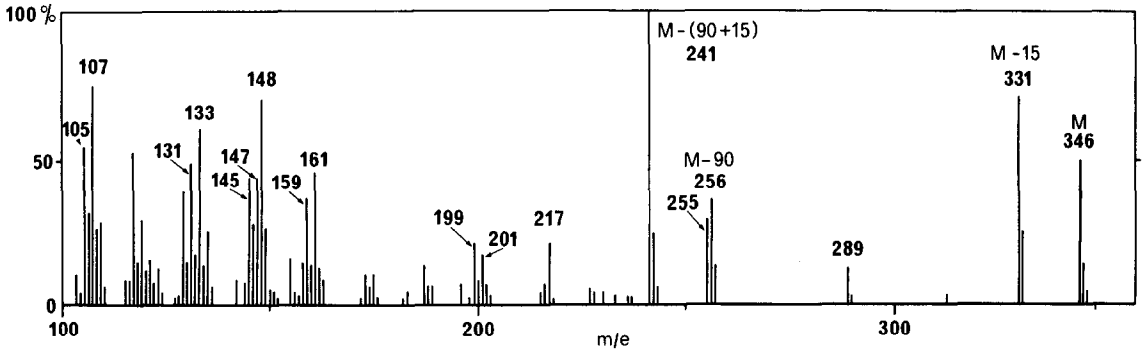

Fig. 1. Mass spectrum of the TMS ether derivative of the compound in rat testis identified as 5α-androst-16-en-3β-ol.

Table 2
Concentrations of endogenous neutral steroids in rat testis. The values are expressed as μg/100 g tissue wet weight.

Sample	Sample weight (g)	Testosterone	Pregnenolone	5α-Androst-16-en-3β-ol
Testis	245.6	10.6	0.8	2.8
Interstitial tissue	2.85	55.0	3.3	*
Tubules	32.8	13.1	0.54	1.1
Cryptorchid testis	17.6	13.5	0.7	4.6

* Sample lost.

although sulphate conjugated steroids have a prominent position in the testes of man and boar. It has been suggested that in rat testis steroid sulphatase might play a role in the regulation of testosterone biosynthesis [1, 2]. In the light of the present results, this does not seem very likely and is in accord with a recent report on testis steroid sulfatase activity in rats treated with chorionic gonadotropin [14].

Recently, Cooke et al. [15] reported endogenous testosterone concentrations in rat testis interstitial tissue and seminiferous tubules. Their results are consistent with those in table 2 demonstrating that the highest concentrations of testosterone were found in the interstitial tissue. The testosterone concentrations found in this study are about 3-fold and 6-fold greater than those reported by Cooke et al. [15] in the interstitial tissue and tubules, respectively. The specificity of the quantitative measurements in the present investigation was confirmed by repetitive mass spectrometric scanning of the column eluate. No contaminating compounds were detected during the elution of the compounds measured.

According to the concentrations of pregnenolone, it is primarily formed in the interstitial tissue (table 2). The same seems to be true in the case of 5α-androst-16-en-3β-ol, because its concentration is higher in cryptorchid testes than in total testis and in interstitial tissue. All the three steroids detected have access to tubular tissue.

The present results do not allow any conclusions concerning the pathways of testosterone biosynthesis in rat testes. *In vitro* studies have demonstrated that rat testis tissue is able to convert certain precursors into 16-unsaturated derivatives, albeit to a small extent (summarized in [16]). The formation of one such compound, 5α-androst-16-en-3β-ol, was demonstrated in this study to take place *in vivo*.

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